1

CHEMICAL CONSTRUCTS

Field of the Invention

This invention relates to chemical constructs for use in solid phase synthesis, and to methods of detecting and/or characterising the products of solid phase synthesis using the constructs.

Background of the Invention

Solid phase synthesis has been known for many years in the field of peptide synthesis and more recently has also been used increasingly for the synthesis of non-peptides.

Solid phase synthesis has found particular application in the field of combinatorial chemistry and the preparation of chemical libraries as potential sources of new leads for drug discovery, see for example Anthony W. Czarnik, *Analytical Chemistry News and Features*, June 1, 1998, pp 378A-386A, and *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998. A feature of combinatorial chemistry methods is that they enable very large numbers of different compounds to be prepared from a relatively limited number of molecular building blocks in a relatively small number of reactions. Combinatorial chemistry makes use of the "split and pool" approach in which a suspension of chemical starting material tethered to a solid support is split into N portions, each of which is reacted with a different reagent. The products of the N reactions are then pooled and mixed thoroughly, the resulting pool is split into N' portions and again each portion is reacted with a different reagent. This procedure can be repeated as many times as there are steps in the reaction sequence. Thus, for a three step reaction sequence, if the reaction mixture is divided into ten portions at each stage, each of the pools being reacted with a different reagent before recombining with the other portions, the total number of compounds formed by the process will be $10^3 = 1000$. Thus it can be seen that by using the split and pool technique, a large number of different molecules can be synthesised using a minimal number of reactions (thirty in the case mentioned above).

Each of the solid supports (e.g. a resin bead) will have a single product molecule

tethered to it and hence in principle each of the reaction products can be separated and analysed or subjected to biological testing simply by isolating each single solid support and cleaving the product from the support. However, the large numbers of compounds generated by combinatorial methods means that it can be impracticable to identify and characterise each compound. Consequently, the compounds usually are first tested, either on the solid support or after cleaving from the support, and only those compounds, which show some biological activity, are subsequently identified. In order to minimise the number of biological tests carried out, compounds can be tested in pools containing a predetermined number of compounds, the inactive pools being discarded and the active pools being subject to further investigation. The biological activities of the compounds can be analysed using high throughput automated assay techniques permitting large numbers of compounds to be analysed in a short time.

The resin beads typically used in the synthesis of combinatorial libraries are derived from cross-linked polystyrene resin and are usually of the order of 90μm and 250μm in diameter (i.e. just visible to the naked eye). The number of beads in a given mass or volume of resin will depend on the average bead size but, for example, with a bead size of 150μm diameter, there will be approximately 500 beads per milligram of bulk resin. Typical compound loadings on the beads are of the order of 0.1 to 0.4 mmol g^{-1} and hence beads of the dimensions given above will tend to have individual compound loadings of about 1 nanomole of compound per bead. It will be appreciated therefore that one of the problems facing the chemist working with solid phase constructs is how to identify and characterise the various compounds in a combinatorial library since each compound may be present in the library in very small amounts, and there will usually be insufficient compound present on a given solid support to allow for both biological testing and identification of the compound.

One known approach to the problem of how to identify the compounds in a combinatorial library is to provide each solid support with a coding tag from which the identity of the compound can be determined. Thus, for example, a coding tag can be built up in sequential steps on the solid support in parallel with the construction of the desired target compound, the coding tag reflecting the synthesis history of the product compound and being unique for each product compound. The coding tag is usually built up on the support using chemical reactions of a type which are orthogonal to the chemistry used to build up the product compound, thereby ensuring that the coding units and product compounds do not become confused. Once the product compound has been tested, and its biological activity confirmed, the coding tag can then be decoded to allow identification of the compound.

In an alternative approach, as described in Geysen *et al.*, Chemistry & Biology, 1996, Vol. 3, No. 8, pp 679-688, and in WO 97/37953, coding tags such as isotopic labels can be incorporated into *inter alia* a linker group between the solid support and the substrate. This approach has the advantage that it does not require the formation of a separate coding tag using orthogonal chemistry and thereby reduces the overall number of synthesis steps involved.

A further problem facing the solid phase chemist is the difficulty in quantifying the products of a given reaction sequence. In any solid phase synthesis procedure, whether or not part of a combinatorial procedure, it is important to be able to determine the optimal conditions for a given reaction step in a series of steps. It is also important to be able to monitor the progress of a reaction so that it can be determined whether a particular reaction has gone to completion. This is particularly important in a multistage solid phase synthesis where the failure of a given stage to proceed to completion can lead to the formation of side products thereby complicating what is otherwise a relatively straightforward separation procedure. Where multiple products are formed in a given reaction step or sequence of steps, or where a given reaction step has not proceeded to completion, it can be extremely useful to have means of quantifying the reaction products, either in absolute terms, or in terms of the relative concentrations with respect to other reaction products. Where the products are produced in milligram quantities, quantitation can be carried out using a variety of instrumental techniques, for example nuclear magnetic resonance (nmr) spectroscopy. However, quantitation is much more difficult where the amount of product available for analysis may be as little as 1 nanomole.

Although both destructive and non-destructive analytical methods are known for determining the products of solid phase synthesis (see *The Combinatorial Index, idem*), one of the recognised problems with solid phase synthesis is that it is generally more difficult to monitor reactions carried out on solid supports than it is to monitor conventional solution phase reactions; see for example the Czarnik paper referred to above. This is particularly true with the products of combinatorial chemistry methods where there is a need to use rapid high throughput techniques to analyse the large numbers of compounds generated by such methods. Techniques such as mass spectrometry are potentially ideally suited as a means of providing high throughput analyses, but a substantial problem is that not all compounds produce a guaranteed response under mass spectrometric conditions. Indeed, many compounds are "invisible" to the so-called "soft" methods of mass spectrometry such as Matrix-Assisted Laser-Desorption Ionisation (MALDI) and Electrospray mass spectrometry which are intended to

detect molecular ions without causing significant fragmentation of the molecule. In this context, the term "soft " means mass spectral methods that give molecular ions and not merely fragments. One of the reasons for this is that under MALDI and electrospray conditions, many compounds, particularly peptides, do not ionise sufficiently well to give a detectable mass

5   spectral response.

Geysen et al. (idem) address this problem by suggesting the introduction of a readily ionisable basic centre such as lysine into the linker, and further suggest that the basic centre could be quaternised by alkylation during work up in order to give a charged group which

10   would sensitise the construct to mass spectrometric analysis. However, one drawback of this approach is the need to protect the amine group during the various synthesis steps and the consequent need to deprotect the amine group prior to analysis.

A development of the Geysen approach is disclosed in Carrasco *et al. Tetrahedron*

15   *Letters*, 1997, 38, No. 36, pp 6331-6334. Carrasco's method involves the formation of a construct comprising a resin bead linked via a first linker group to a group, which the authors refer to as an "ionisation tag". The "ionisation tag" in turn is linked via a second linker group to a substrate. The first and second linker groups are orthogonally cleavable; i.e. they can be selectively cleaved using different chemistries.

20

In the specific example disclosed in Carrasco *et al.*, the first linker group is photochemically cleavable whilst the second linker group is chemically cleavable. The "ionisation tag" used by Carrasco is a tetrapeptide chain having the sequence (reading from the C-terminus) Gly-Phe-Lys-Ala, and having an N-(2-trimethylammonium)-acetyl group linked to

25   the lysine. The purpose of the ionisation tag is twofold. Firstly, it provides an already ionised "sensitiser group" which enables the construct to be detected by matrix-assisted laser-desorption ionisation (MALDI) mass spectrometry, and secondly, it adds mass to the construct thereby enabling substrate molecules to be detected without being swamped or masked by low molecular weight peaks in the mass spectrum.

30

However, there are several significant potential problems with the Carrasco constructs and in particular the "ionisation tag" used in the construct. More particularly, it is envisaged that the quaternary ammonium sensitiser group will be incompatible with many of the types of chemistry that the chemist might wish to perform on the constructs. Thus, for example, the

35   trimethylammonium group is potentially chemically reactive and, furthermore, the cationic group may function as an ion exchange centre and complex with anions.

Summary of the Invention

It is an object of the present invention to provide a means of monitoring chemical
reactions on solid supports which avoids problems inherent in known methods and which
provides a means of detecting and identifying the products of solid phase synthetic techniques
using mass spectroscopy.

Accordingly, in a first aspect, the invention provides a chemical construct for use in
solid phase synthesis comprising a solid support Q having linked thereto via a connecting group
Y a substrate R; the connecting group Y having first and second cleavage sites which are
orthogonally and selectively cleavable; the second cleavage site being selectively cleavable to
release the substrate; and the first cleavage site being located at a position between the second
cleavage site and the solid support and being selectively cleavable to release a fragment Fr
comprising the substrate and at least a portion of the connecting group Y; characterised in that
cleavage at the first cleavage site forms or introduces on the chemical fragment Fr at the first
cleavage site a moiety comprising a sensitising group G which sensitises the chemical fragment
Fr to instrumental, e.g. mass spectroscopic analysis.

In the constructs of the invention, the sensitising group G is formed or introduced by
cleavage of the "skeleton" of the construct. This is in contrast with known constructs (see for
example Carrasco *et al., idem*), where the sensitising group is present throughout the synthesis
of the substrate, or where the sensitising group is revealed by cleavage of a side chain or
removal of a protecting group from a pendant sensitising group.

The sensitising group G renders the fragment Fr, and hence indirectly the substrate R,
more sensitive to analysis by a given analytical technique, and in particular a mass
spectrometric technique. Thus the sensitising group can be a group which is readily ionisable
under the conditions encountered in a mass spectrometer, and in particular electrospray mass
spectrometry, to afford a strong signal.

The ionisable sensitising group formed during the selective cleavage from the solid
support of the chemical fragment Fr serves to ensure that the fragment is ionised sufficiently in
the mass spectrometer to give a strong response. This overcomes a problem inherent in many
molecules synthesised by solid phase methods where a suitable ionising group is not present
and analysis by high throughput mass spectral techniques is problematical.

6

The ionisable group can be for example a basic amino group or a carboxylate group but preferably it is a basic amino group. It will be appreciated that the term "basic amino group" as used herein refers in particular to an amino group that is readily protonated.

The basic amino group can be a primary amino group, a secondary amino group, or a tertiary amino group. Where the basic amino group is a tertiary amino group, it can be for example, a cyclic amino group such as piperidino, piperazino, pyrrolidino, or morpholino, piperidino or piperazino (e.g. N-methylpiperazino) being presently preferred.

In accordance with the invention, a moiety comprising the sensitising group G, such as a basic amino group, is formed or introduced at the first cleavage site as the chemical fragment Fr (hereinafter referred to as the analytical fragment) is cleaved from the solid support. The advantage of forming the sensitising group G in this way is that it avoids the potential problem of a pre-existing or pre-formed sensitising group interfering with the chemistry of the construct. Moreover, it avoids the need to have the sensitising group independently protected thereby obviating the additional problem of de-protection, a requirement which could limit the types of chemistry available to the chemist for cleavage at the first and second cleavage sites. Thus, for example, if a protected pendant sensitising group were present, and for example the conditions required for de-protection involved the use of an acid such as trifluoroacetic acid, then this would effectively prevent the use of an acid labile cleavage site as the second cleavage site linking the substrate to the rest of the construct. The constructs of the invention avoid such problems.

The atoms or functional group making up the sensitising group G can be present in a masked form in the construct before cleavage of the fragment Fr from the resin, the cleavage conditions merely serving to unmask the sensitising group G. Alternatively, the atoms or functional group making up or containing the sensitising group G can be introduced at the cleavage site during the cleavage reaction. For example, where the sensitising group is a basic amino group, the nitrogen atom of the amino group can be present in the construct before cleavage, or it can be introduced during the cleavage reaction.

When the sensitising group G is introduced from an external source during the cleavage reaction, it can form part of a larger chemical moiety. For example, the group G can be introduced as part of a group X-G wherein X is the residue of a nucleophile or electrophile, for example a nitrogen or sulphur-based nucleophilic group, e.g. a group of the formula G-Alk-

7

Nuc wherein Alk is an alkylene group (e.g. a C_{2-20} and preferably C_{2-6} alkylene group) optionally interrupted by one or more heteroatoms selected from oxygen, nitrogen and sulphur, and Nuc is a nucleophile, such as an amine (e.g. NH or NR' where R' is a C_{1-6} alkyl group) or a thiolate group., and Nuc is a nucleophile, such as an amine or a thiolate group.

It is preferred that the chemical fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment. The signature can advantageously be provided by incorporating into the fragment a "peak splitting" isotopic label. The peak splitting isotopic label comprises at least one atom that exists in a number of stable isotopic forms. By isotopic labelling of one or more particular atoms in the fragment Fr such that a given atom is labelled with a mixture of isotopes, the mass spectra of the molecular ions will appear as a characteristic pattern, the precise pattern depending on the relative amounts of the individual isotopes. Thus, for example, if a given atom in the fragment Fr is labelled such that 50% of the atoms are of one isotopic form and 50% are of another isotopic form, the mass spectrum will show the molecular ion as a characteristic doublet in which the peaks are of approximately equal height.

The purpose of the peak splitting atom(s) is to provide a characteristic pattern that will characterise any peak in the mass spectrum originating from the analytical fragment Fr, thereby distinguishing such peaks from those due to extraneous materials.

Examples of atoms that can be used as isotopic peak splitting labels include $^1H/^2H$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

The fragment Fr can contain a single peak splitting isotopic label or more than one such label. For example, the isotopic label can be a single bromine atom in which case the peak for the molecular ion of the analytical fragment Fr liberated following cleavage from the solid support will appear as a doublet. By introducing a second or subsequent peak splitting label(s), a more complex peak pattern will be produced for the molecular ion.

The isotopic peak splitting label(s) preferably is/are located between the first and second cleavage sites.

The first and second cleavage sites can be defined by first and second linker groups L^1 and L^2. A "spacer group" A can be interposed between the two linker groups L^1 and L^2, the spacer group A typically containing an isotopic peak splitting label. Accordingly, in one

8

preferred embodiment of the invention, there is provided a chemical construct as hereinbefore defined wherein the connecting group Y has the formula L^1-A-L^2; wherein L^1 is a first linker group defining the first cleavage site; A is a chemical group (the spacer group) containing a peak splitting isotopic label; and L^2 is a second linker group defining the second cleavage site.

The first and second cleavage sites are orthogonally and selectively cleavable; i.e. the conditions used to effect cleavage at one of the cleavage sites will not cleave the other. A wide variety of different types of cleavage reaction can be used, examples being reactions selected from acid catalysed cleavage, base catalysed cleavage, metal catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, cleavage by 1,2 *bis* nucleophiles, electrophilic displacement, and thermal, photochemical and enzymatic cleavage.

Examples of linker groups that can be selectively cleaved under photochemical conditions to reveal or introduce a sensitising group G (e.g. an amine group) include carbamate groups such as *o*-nitrobenzylcarbamate groups in which initial cleavage takes place between the benzylic methylene group and the adjacent oxygen atom, followed by loss of carbon dioxide to give a basic amino group.

Examples of linker groups that can be cleaved by nucleophilic displacement (e.g. to reveal or introduce a sensitising group G) include sulphonamide linker groups in which the aryl (e.g. phenyl) ring of the sulphonamide contains an electron withdrawing group such as a nitro group, preferably in the *ortho* position of the aryl ring (see for example Kay *et al.* Tetrahedron Letters - 1997, 38(39)). Cleavage of the linker between the amino and sulphonyl moieties can be effected by a nucleophile such as a thiol or thiolate nucleophile in the presence of a base such as 1,8-diazobicyco[5.4.0]undec-7-ene (DBU), morpholine or sodium carbonate. Further examples of groups that can be cleaved by nucleophilic displacement include mercaptopyrimidine-based "safety catch" linkers such as 5-carboxy-2-mercaptopyrimidine, where cleavage can be effected by reacting under oxidising conditions to generate a sulphoxide or sulphone linkage, followed by reaction with a nucleophilic amino group to form a 2-aminopyrimidine. Such linkers can be cleaved, for example, by reaction with a cyclic amine such as piperidine or an N-substituted piperazine (e.g. N-methylpiperazine), or an amino group-containing thiolate nucleophile (e.g. dimethylaminoethylthiolate) after first oxidising with an oxidising agent, preferably a relatively mild oxidising agent such as a per-acid or an inorganic per-salt such as potassium peroxymonosulphate (e.g. "Oxone").

Either the first or second cleavage sites/linkers, or both, but preferably the first, can be of the

9

"safety catch" variety; i.e. the cleavage site or linker group must be chemically modified in a first step before it can be subjected to cleavage in a second step (for example to reveal or introduce the sensitising group G). An advantage of such an arrangement is that it prevents or significantly reduces the possibility of cleavage taking place inadvertently. One example of a "safety catch" mechanism involves oxidation of a functional group (e.g. the mercaptopyrimidine-based "safety catch" linker described above) in a first step, the oxidation serving to make the functional group more amenable to displacement by a nucleophile in a subsequent cleavage step. The nucleophile can vary considerably in structure. For example, in one embodiment, the nucleophile can be an amino group-containing nucleophile), the amino group participating in the nucleophilic displacement action such that the amino group (sensitising group G) is attached directly to the cleavage site. Alternatively, in another embodiment, the amino group (or other sensitising group) can be present in a group (e.g. a dialkylaminoalkyl-thiolate anion) containing another nucleophile such as a sulphur nucleophile that becomes attached to the cleavage site.

Another class of linker groups suitable for use in the constructs of the present invention is the class of linker groups that are cleavable by 1,2 *bis* nucleophiles such as hydrazines and hydroxylamines. Such *bis* nucleophiles can be used to cleave the bond between the nitrogen atom and adjacent carbon atom in certain enamine systems, for example enamine systems in which the enamine is conjugated to a carbonyl group. Examples of such groups are groups based on oxo-substituted cycloalkylidene rings such as the 1-[4,4-dimethyl-2,6-dioxo-cyclohexylidene]-ethyl (dde) group coupled to an amino nitrogen atom. The constructs are preferably set up such that the enamine system defines the first cleavage site, breaking of the bond between the nitrogen atom and adjacent carbon atom as described above revealing an amine group that functions as a mass spectrometric sensitiser.

Either the first or second, but preferably the first, cleavage sites can be defined by linker groups that are cleavable under the action of transition metals, preferably palladium (0). Examples of such linker groups include allyloxy carbonyl amino groups which, when cleaved, release an amine group capable of acting as a mass spectrometric sensitiser.

Examples of linkers that can be selectively cleaved under acidic conditions include appropriately substituted benzyloxycarbonyl groups and appropriately substituted diphenyl-methylamino groups, both of which can be cleaved by the action of trifluoroacetic acid.

Particular examples of acid cleavable linker groups are set forth in *The Combinatorial*

Index, Barry A. Bunin, Academic Press, San Diego 1998, the disclosure of which is incorporated herein by reference. Linkers of the "Rink" or "Knorr" type typically comprise an N-protected 1-amino-1,1-diphenylmethane moiety, the amino group when deprotected allowing attachment to a substrate, one of the phenyl rings being substituted for example with dimethoxy groups and the other having a carboxyalkyloxy substituent providing a second point of attachment. Cleavage with TFA gives rise to a substrate compound having a terminal carboxamido group. Linkers of the "Wang" type typically contain a substituted phenoxyacetyl group, the acetyl group providing one point of attachment, and a benzylic hydroxyl group on the phenyl ring forming a second point of attachment. Esters can be formed between a carboxyl group of a substrate and the benzylic hydroxyl group, the ester groups being subsequently cleavable with TFA to release a substrate compound having a terminal carboxylate group.

Examples of linkers that can be selectively cleaved under basic conditions include groups containing ester linkages.

In one embodiment, there is provided a chemical construct as hereinbefore defined wherein the first cleavage site is selectively cleavable to form or introduce a sensitising group G (in particular an amino group) and the cleavage is effected by one type of chemistry selected from a group of chemistries consisting of cleavage under acid conditions, base catalysed cleavage, palladium (0) catalysed cleavage, oxidative cleavage, oxidation followed by nucleophilic displacement, reductive cleavage, nucleophilic displacement, cleavage by 1,2-*bis* nucleophiles, electrophilic displacement, and thermal, photochemical and enzymic cleavage, and the second cleavage site is selectively cleavable by a different type of chemistry selected from the said group.

Although groups that are cleavable under acidic conditions can be used to form the first cleavage site, it is currently preferred that the first cleavage site is stable to acidic conditions. However, the second cleavage site may advantageously be cleavable in acidic conditions.

In a preferred embodiment, the first cleavage site is cleavable to form or introduce the sensitising group G, the cleavage being effected by one type of chemistry selected from:

       (i)   photochemical cleavage, e.g. photochemical cleavage of a carbamate group such as a nitrobenzylcarbamate group;

      (ii)   oxidation followed by cleavage through nucleophilic displacement, for example oxidation of a thiopyrimidine followed by nucleophilic displacement

by an amine (e.g. a secondary amine such as N-methyl piperazine);

(iii)   cleavage of a sulphonamide by nucleophilic displacement, for example by a thiolate nucleophile (e.g. mercaptoethanol in the presence of a strong base such as DBU);

(iv)   cleavage of enamine groups (particularly those containing an enamine moiety conjugated to a carbonyl group; e.g. as 1-[4,4-dimethyl-2,6-dioxo-cyclohexylidene]ethyl amino) with a 1,2-*bis* nucleophile such as hydrazine or hydroxylamine or derivatives thereof; and

(v)   transition metal catalysed cleavage of allyloxycarbonylamino groups, for example palladium (0) catalysed cleavage of allyloxycarbonylamino groups.

In the aforementioned preferred embodiment, the second cleavage site can be, for example, cleaved under acid conditions or by photolysis.

In one preferred embodiment, the first cleavage site is defined by a sulphonamide linker group, and the second cleavage site is optionally defined by a group, such as a Rink linker, which is cleavable under acidic conditions.

In another preferred embodiment, the first cleavage site is defined by a thiopyrimidine linker susceptible to cleavage by oxidation followed by nucleophilic displacement, and the second cleavage site is optionally defined by a group, such as a Rink linker, which is cleavable under acidic conditions.

In a further preferred embodiment, the first cleavage site is defined by a dde group as hereinbefore described, and the second cleavage site is optionally defined by a group, such as a Rink linker, which is cleavable under acidic conditions.

In a still further preferred embodiment, the first cleavage site is defined by a photochemically cleavable carbamate group as hereinbefore described, and the second cleavage site is optionally defined by a group, such as a Rink linker, which is cleavable under acidic conditions.

In yet another preferred embodiment, the first cleavage site is defined by a group such as allyloxycarbonylamino that can be cleaved by a transition metal such as palladium (0), and the second cleavage site is optionally defined by a group, such as a Rink linker, which is cleavable under acidic conditions

12

By making the first and second cleavage sites, e.g. as defined by the first and second linker groups L^1, L^2, orthogonally cleavable, it is possible selectively to separate from the construct either the chemical fragment Fr or the substrate R, simply by using different cleavage conditions. This means that during experiments designed to optimise the conditions for a
5   particular reaction step, the chemist can subject the construct to conditions suitable for cleaving off the analytical fragment Fr thereby allowing analysis to be carried out to determine the outcome of each test reaction. Similarly, during a preparative reaction (e.g. preparation of a combinatorial library or subsequent preparative reactions such as scale-up reaction or commercial production), quality control (QC) can be carried out by removing one or more solid
10   supports from the reaction vessel, cleaving the constructs at the first cleavage site and analysing the resulting fragment Fr to see whether a particular reaction step has worked. On the other hand, by cleaving at the second cleavage site e.g. on the second linker group, rather than the first, the reaction product R is released from the solid support. Thus, an advantage of the constructs of the present invention is that they can be used both at an experimental level to
15   optimise a particular process step, and also at a preparative level without modifying the linker groups.

In one embodiment of the invention, the fragment Fr, and more preferably the spacer group A contains an alkylene diamine group or aminoalkoxy group. The precise size of the
20   alkylene group and its degree of substitution is not currently considered to be important, but by way of example the chain could be from 2 to 30 carbon atoms in length, for example up to 20 carbon atoms, more typically less than 10 carbon atoms, particular examples being ethylene or propylene diamine or aminoalcohol groups which may be substituted or unsubstituted. The alkylene diamine group or aminoalcohol typically contains a peak splitting isotopic label as
25   hereinbefore defined. The two amino groups can each be bonded to respectively the first and second linker groups. In order to increase the mass of the spacer group A, the alkylene diamine group can be substituted by an aryl group, or example an N-aryl group such as an N-benzyl group. The N-aryl group may optionally be substituted with one or more substituent groups.

30   Where, as is preferred, the spacer group contains one or more mass-spectral peak splitting isotopic labels, these can be located either in the alkylene chain or in a substituent group attached to the alkylene chain. Thus, for example, an N-benzyl group bonded to one of the two amino groups in an alkylenediamine can have a methylene group that is substituted with the peak splitting atom deuterium. Alternatively, an aryl ring (e.g. an N-benzyl group)
35   present in a substituent on the alkylene diamine can be substituted with a peak splitting bromine atom, one particular example of an aryl group being an N-o-bromobenzyl group.

Although alkylene diamine and amino alcohol groups are given as specific examples of spacer groups, alternative spacer groups can be used. For example, the spacer groups can be formed from hydrocarbon chains containing up to thirty or more carbon atoms in the chain which can be optionally interrupted with one or more heteroatoms such as oxygen, nitrogen or sulphur. As a further alternative, the spacer can be for example a peptide chain containing one or more amino acids. The precise nature and length of the spacer is not currently considered to be important provided that the spacer does not interfere with the chemistry of the construct.

The chemical fragment Fr, and in particular the spacer group A, as well as providing a means of identification using mass spectrometry, can also be provided with one or more additional sensitisers to allow characterisation by other analytical techniques. For example, the spacer group can contain a chromophore to allow characterisation by ultra violet (u.v.) or fluorescence spectroscopy.

More particularly, in order to allow for quantitative analysis to be carried out to determine either the relative amount (e.g. relative to another substrate R) or absolute amount of substrate present in a reaction mixture, the connecting group Y and fragment Fr can contain a chromophore C^u which facilitates analysis of the fragment Fr by ultraviolet, visible or fluorescence spectrophotometry, and preferably by ultraviolet spectrophotometry.

Accordingly, in another aspect, the invention provides a method of analysis of a solid phase construct; which method comprises:

(i)   providing a chemical construct comprising a solid support Q having linked thereto via a connecting group Y a substrate R wherein Q, Y and R are as hereinbefore defined; the connecting group Y having first and second cleavage sites which are orthogonally and selectively cleavable as hereinbefore defined; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectrophotometry (preferably UV spectrophotometry);

(ii)   cleaving the connecting group at the first cleavage site to release the fragment Fr; and

(iii)   subjecting the fragment Fr to ultra violet, visible or fluorescence spectrophotometric analysis to quantify the substrate R.

In accordance with the foregoing method aspect of the invention, the spectrophotometric analysis may be used to determine either the absolute amount of substrate R present in a given sample, or it may be used to determine the relative amount (e.g. in terms of a molar ratio) of substrate R present relative to another component (e.g. where more than one substrate R is present) in the sample. References to methods of quantifying the substrate R in this application include both absolute determination and relative determination, unless the context indicates otherwise.

In order to ensure that any chromophores present in the substrate R do not interfere with the analysis, the chromophore is typically selected such that it has a substantial absorption band that distinguishes it from the substrate. Thus, for example, the absorption band may be remote from any significant absorption bands in the substrate. Alternatively, the absorption band in the chromophore may be of such an intensity that it effectively swamps any overlapping band in the substrate. It is preferred that the chromophore C^u has a principal log E_{max} value of at least 2.5. It is further preferred that the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R.

The relationship between the absorption of visible or UV radiation by a compound in solution and the concentration of the compound is defined by the Beer-Lambert law which can be expressed by the formula $E = A/cl$ where A is the absorbance or optical density of a sample solution, c is the molar concentration, l is the path length of the sample, and E is the molar absorptivity. The molar absorptivity E is constant for a given compound at a given wavelength and is usually expressed as E_{max} - the molar absorptivity at an absorption band maximum. The values for E or E_{max} are typically expressed in units of 10^{-2} m^2 mol^{-1} and, unless indicated otherwise, references to E or E_{max} values herein will refer to such units. Since E or E_{max} values can be very large, the logarithmic equivalent is often used and such logarithmic equivalents are referred to herein as log E_{max} values.

A given compound will often have significant absorption peaks or bands at several different wavelengths and hence can often be defined with reference to several E_{max} values. In the context of the present invention, the term "principal E_{max} " is used to denote the E_{max} at the wavelength at which the greatest absorbance occurs. Thus, in the constructs of the present invention, the chromophore C^u preferably has a principle log E_{max} value of at least 2.5 and typically has a principal log E_{max} value which is at least 1.5 times greater than the principal E_{max} of the substrate R. More typically however, the chromophore C^u has a principal log E_{max} that is

at least 2, more usually 2.5, and preferably 3 times greater than the principal log E_{max} of the substrate R.

Although analysis of the compounds can be effected by measuring absorbance at a principal maximum, measurement of side bands or lesser maxima may be used instead to determine the amount of compound present. In general, the wavelength at which the measurement is taken will depend on the precise absorption characteristics of the substrates R and the chromophore C^u, and can be determined on a case by case basis.

The chromophore C^u may be distinguished from any chromophores in the substrate R in that it may possess an absorption band which is remote from any significant absorption bands. By this is meant that the absorption band of the chromophore is remote from, and does not overlap to any significant extent (e.g. less than 5% overlap with respect to the area under the curve (a.u.c.) of the respective absorption peaks) with any significant absorptions due to the substrate R. A significant absorption in the present context means an absorption having a log E value of 1 or more.

In one preferred embodiment of the invention, there is provided a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R wherein Q, Y and R are as hereinbefore defined; and the connecting group Y is orthogonally and selectively cleavable as hereinbefore defined; wherein the fragment Fr comprises the substrate and at least a portion of the connecting group Y, and the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, the chromophore C^u having a principal log E_{max} value of at least 2.5 and wherein (i) the principal log E_{max} value is at least 1.5 times greater than the principal log E_{max} of the substrate R; or (ii) , the chromophore C^u has an absorption peak at a wavelength remote from absorptions due to the substrate R.

The presence of a UV chromophore in the construct enables quantitation of the products of a synthesis to be carried out. Such quantitation can be (i) absolute quantitation or (ii) relative quantitation, or both. By absolute quantitation is meant that the absolute amounts of a substrate molecule or fragment or construct containing the substrate molecule can be determined, whereas the term " *relative quantitation*" is used herein to mean the determination of the amount of a substrate (or fragment or construct containing the substrate) relative to another component (such as a side product or starting material or another substrate) in a reaction mixture.

16

The UV chromophore is typically selected such that it has a very strong absorption at a unique or characteristic wavelength, which is usually distinct from the wavelengths at which the maximum absorbances of a typical substrate molecule might be found. However, in cases where the absorption of the chromophore is very large relative to the substrate or substrate R, the overlapping of absorptions due to the substrate and the chromophore may have a minimal impact on the accuracy of the measurement of the quantity of substrate. In general, overlapping absorption bands should not prevent meaningful analyses to be carried out provided that any error introduced is no greater than 10% and preferably no greater than about 5%. Accordingly, as an alternative to defining the chromophore in terms of relative absorbances and absolute molar absorption values, the chromophore C, and its relationship with the absorption due to the substrate can be defined in terms of the degree of error arising from any overlap between the absorption bands of the substrate R and the chromophore.

Thus, in a further embodiment, the invention provides a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto via a connecting group Y a substrate R wherein Q, Y and R are as hereinbefore defined; and the connecting group Y is orthogonally and selectively cleavable as hereinbefore defined; wherein the fragment Fr comprises the substrate and at least a portion of the connecting group Y, and the said portion contains a chromophore C^u which facilitates analysis of the fragment Fr^u by ultra violet, visible or fluorescence spectroscopy, wherein the absorption characteristics of the chromophore C^u and the substrate R are such that at a given measurement wavelength, any errors in measurement of the quantity of substrate R (or any fragment or construct containing the fragment) arising from any overlap between absorption bands due to the chromophore and absorption bands due to the substrate R are less than 10%, preferably less than 5%.

Examples of chromophores are groups containing an aryl group, preferably a fused polycyclic aryl group, e.g. a C_6 - C_{30} polycyclic aryl group in which one or more (e.g. 1, 2, or 3) ring carbon atoms are optionally replaced by a heteroatom such as nitrogen, sulphur or oxygen. Examples of polycyclic aryl groups are polycyclic hydrocarbons such as naphthyl, phenanthrenyl and anthracenyl groups, and polycyclic heteroaryl groups such as acridine, or phenanthroline. Such aryl groups or polycylclic groups can be optionally substituted, preferably with a substituent or substituents which is or are inert and/or non-interfering with regard to the reaction conditions employed in a given reaction scheme.

Examples of such substituent groups include alkyl and alkoxy (e.g. alkyl and alkoxy

having from 1 to 20 carbon atoms, preferably from 1 to 6 carbons), each optionally containing one or more unsaturated linkages, and being optionally interrupted by one or more heteroatoms selected from oxygen, nitrogen and sulphur; amino (preferably protected amino or mono-or disubstituted amino, e.g. dimethylamino); halogen such as fluoro, chloro, bromo and iodo;

5   alkylthio (e.g. C_{1-20} alkylthio, preferably C_{1-6}), hydroxy, protected (e.g. acylated) hydroxy, thio, protected (e.g. acylated) thio, trifluoromethyl, nitro, alkylsulphonyl, aryl (e.g. optionally substituted phenyl, thienyl, furanyl), and arylalkyl (e.g. benzyl and phenethyl).

Other examples of chromophores include highly conjugated aryl or non-aryl systems,

10   provided that such compounds are inert under the synthetic conditions employed in the solid phase chemistry, and so-called "push-pull" systems of the type G-J-M wherein G is an electron-donating group (e.g. amino or alkoxy), J is an conjugated array of multiple, e.g. double, bonds, and M is an electron withdrawing group (e.g. nitro or sulphonyl), the groups G and M being electronically linked through the conjugated array of multiple bonds. One example of such as

15   chromophore is the dansyl (1-dimethylamino-5-naphthylsulphonyl) group.

The precise nature of the chromophore, its chemical properties and its absorbance characteristics, can be determined on a case by case basis depending upon the type of chemistry to be used in a given synthesis. It is most preferred however, that the chromophore is inert to

20   the reactants and reaction conditions employed in the synthesis.

Presently preferred chromophores include anthracenyl and dansyl (5-dimethylamino-1-naphthylsulphonyl groups, anthracenyl being particularly preferred.

25   The quantitation of the substrate R can be either absolute quantitation or relative quantitation in which the relative amounts of the substrate R and another component in the synthesis mixture (e.g. another substrate, or starting material, or a side product for example) are determined. Relative quantitation makes use of the fact that the chromophore C^u confers on each substrate a common set of characteristic absorbances that can be used as the basis for the

30   spectrophotometric analysis.

In one embodiment of the invention, the characterisation and/or analysis of the constructs of the invention is carried out on a single bead, or a small number of beads, for example from 1 to 20 beads, more usually less than ten beads. Such beads typically have an

35   average diameter in the range from 90μm to 250μm and a compound loading of between 0.1 mmol g^{-1} and 0.4 mmol g^{-1}average loading of an individual bead is less than 10 nanomoles,

18

often less than 5 nanomoles, for example approximately 1 nanomole.

Accordingly, in one particularly embodiment, the invention provides a method of analysis of a solid phase construct; which method comprises:

5   (i)   providing a chemical construct comprising a solid support Q (e.g. a resin bead having an average diameter in the range from 90μm to 250μm) having linked thereto via a connecting group Y a substrate R wherein Q, Y and R are as hereinbefore defined, the substrate R being present on each solid support in an amount of no more than 10 nanomoles, preferably less than 5 nanomoles and more preferably less than 2 nanomoles; the connecting group Y

10   having first and second cleavage sites which are orthogonally and selectively cleavable as hereinbefore defined; the second cleavage site being selectively cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr comprising the substrate and at least a portion of the connecting group Y, wherein the said portion contains

15   a chromophore C^u which facilitates analysis of the fragment Fr by ultra violet, visible or fluorescence spectrophotometry;

         (ii)   isolating a solid support, or a plurality of solid supports not exceeding 20 in number (preferably less than 10, more preferably less than 5, e.g. 1 solid support);

         (iii)   treating the solid support(s) to cleave the connecting group at the first

20   cleavage site to release the fragment Fr^u containing the substrate R; and

         (iv)   subjecting the fragment Fr^u to ultra violet, visible or fluorescence
                    spectrophotometric analysis to quantify the substrate R.

A significant advantage of the use of the UV chromophore-containing constructs of the

25   present invention is that they can be used to provide quantitative information on very small amounts of substrate compounds, for example amounts of the order of 1 nanomolar typically available from single resin beads of the size used in split and mix combinatorial methods. In combinatorial libraries formed by the split and mix method, each bead typically will contain only a single substrate compound, but a library will contain a plurality of beads bearing

30   different substrates. The analysis of mixtures of beads bearing different substrates is problematical and hence it is generally necessary to carry out analyses on single beads. The constructs of the present invention enable analysis to carried out efficiently and accurately at the single bead level, and in particular with beads of a size of the order of 90μm to 250μm in diameter with loadings of the order of 0.1- 0.4 mmol g-1.

35

In constructs where the first and second cleavage sites are defined by first and second

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linker groups L^1 and L^2, and a "spacer group" A is interposed between the two linker groups L^1 and L^2, the spacer group A can contain or have linked thereto the UV chromophore C^u. Thus, in one embodiment of the invention, there is provided a chemical construct as hereinbefore defined wherein the connecting group Y has the formula L^1-A-L^2; wherein L^1 is a first linker group defining the first cleavage site; A is a chemical group (the spacer group) containing or having linked thereto the UV chromophore C^u, and optionally containing a peak splitting isotopic label; and L^2 is a second linker group defining the second cleavage site.

The chromophore C^u may be formed as an integral part of the backbone or skeleton of the fragment Fr^u (e.g. as part of the spacer group A), or it can be a pendant group. Thus, for example, when it is a pendant group, it can be linked to A either directly or via an alkylene chain (e.g. of 1 to 6 carbon atoms) optionally interrupted by one or more oxygen, nitrogen or sulphur atoms, or by means of an ether, thioether, amide, sulphonamide or ester linkage. Where the spacer group A contains an alkylene diamine group or aminoalkoxy group, the chromophore C^u can, for example, be linked (directly or via an optionally interrupted alkylene chain as hereinbefore defined) to the nitrogen atom of the, or one the, amino groups. For example, the chromophore C^u can be linked to the nitrogen atom by means of an ethylene or propylene chain or by a sulphonyl group.

In compounds where a substituent such as a benzyl group is attached to one of the amino groups of an alkylene diamine spacer group A, the chromophore C^u can be linked, directly or indirectly, to the other of the amino groups. In one currently preferred embodiment, the chromophore C^u is a polycyclic aryl group such as an anthracenyl or phenanthrenyl group linked via a propylene chain to a nitrogen atom at one end of the diamine spacer group.

The solid support Q can be any type of solid support suitable for use in solid phase synthesis, and in particular combinatorial chemistry. Thus, purely by way of example, the solid support can take the form of beads, a solid surface, solid substrates, particles, pellets, discs, capillaries, hollow fibres, needles, solid fibres, or organic or inorganic gels such as silica gels, and insoluble organic particles such as particles formed from fullerenes.

Examples of beads are polymeric beads such as cellulose beads or resin beads, particular examples of materials from which resin beads can be prepared including functionalised polymer resins such as polystyrene resins, polyacrylamide resins and dimethylacrylamide resins. Examples of suitable supports are listed in *The Combinatorial Index* by Barry A. Bunin, referred to above. Examples of functionalised resins include cross-

linked polystyrene resins modified to provide amino groups or hydroxyl groups at the surfaces thereof: see for example *Combinatorial Chemistry* by Nicholas K. Terrett, Oxford University Press, 1998.

5   In order to enable to simplify determination of the synthesis history of a solid support, and to provide a means of controlling the mixing of solid supports in split-and-pool combinatorial syntheses, the solid supports (e.g. groups of beads) can be confined within permeable labelled containers throughout a reaction sequence, whereby synthesis takes place as a result of the reactants and solvents flowing through the permeations in the container. A given

10   reaction mixture may contain a plurality of containers and, at the end of each reaction step, the containers can be removed and identified by the label which can be, for example, a radio frequency label. Examples of such containers are the polypropylene mesh "Kan" ™ containers available from Irori of La Jolla, California, USA which contain miniaturised radio frequency tags.

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           In another aspect, the invention provides a method of analysing the constructs hereinbefore defined; the method comprising cleaving the construct at the first cleavage site to release the chemical fragment Fr, the cleavage reaction generating on the chemical fragment Fr a mass spectrometric sensitising group (e.g. a group which is ionisable under mass

20   spectroscopic conditions), and then subjecting the chemical fragment to mass spectrometry, e.g. electrospray mass spectrometry.

           The analysis of the fragment Fr provides information on the reaction history of the construct. Thus, by mass spectrometric analysis, it can readily be determined whether or not

25   the desired substrate has been formed in a given reaction sequence. Analysis of the fragment Fr can therefore be used not only to characterise the substrate or product of the solid phase reaction sequence, but also to follow the progress of the reactions.

           The invention also provides a method of analysing the constructs hereinbefore defined;

30   the method comprising cleaving the construct at the first cleavage site to release the chemical fragment F" and subjecting the fragment to UV, visible or fluorescence spectrometric analysis, for example quantitative UV analysis.

           The cleavage products can be subjected to chromatography, preferably a liquid

35   chromatography technique such as HPLC, using a UV detector which can be set up to provide a means of determining concentrations of the various compounds as they elute from the

chromatography column.   The chromatography column can in turn be linked to another instrumental means of analysis for the purpose of identifying the substrate. A preferred means of instrumental analysis is mass spectroscopic analysis, and thus the method and constructs of the invention afford a means of means of achieving both identification and quantitation of the substrate in a single linked series of analytical procedures

In a further aspect, the invention provides an intermediate chemical construct for use preparing a chemical construct as hereinbefore defined, the intermediate construct having the formula Q-Y' wherein Y' is a reactive or protected form of the group Y, and Q and Y are as hereinbefore defined.

In a still further aspect, the invention provides an intermediate construct of the formula Q-L^1-A^P wherein Q and L^1 are as hereinbefore defined and A^P is a reactive or protected form of the spacer group A containing a splitting isotopic label and optionally a. chromophore C ". In a particular embodiment, the intermediate construct has the general formula Q-L^1-N(Alk - C ")-Alk-NH-X^1 wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group. The intermediate construct is preferably isotopically labelled with a peak splitting combination of atoms such as ^{1}H/^{2}H (D), ^{79}Br/^{81}Br, ^{12}C/^{13}C, ^{14}N/^{15}N and ^{16}O/^{18}O.

The invention further provides a method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as hereinbefore defined and subjecting the library to biological testing to identify biologically active substrates. The method may include the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

Brief Description of the Drawings

Figure 1 shows the mass spectrum of the photolysis product of the construct described in Example 1;

Figure 2 shows the mass spectrum of a fragment produced by cleavage of the thiopyrimidine linker group in the construct described in Example 2;

Figures 3 to 8 show the mass spectra of the photolysis products of the constructs described in Example 4;

Figures 9 and 10 show the mass spectra of analytical fragments produced by palladium (0) cleavage of the "Alloc" - linker - containing constructs of Example 7; and

Figures 11 to 18 show the comparative mass spectra of either a substrate, or an analytical fragment containing the substrate, following cleavage at either the first or second cleavage sites for four of the members of the combinatorial library described in Example 6.

Description of Illustrative Embodiments

The invention will now be illustrated but not limited by reference to the following examples.

In the Examples, the following abbreviations are used.

Abbreviations

AcOH	Acetic acid
DMF	Dimethylformamide
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DIC	Diisopropylcarbodiimide
TFA	Trifluoroacetic acid
DIPEA	Diisopropylethylamine
HATU	2-(1H-9-Azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
DMSO	Dimethylsulfoxide
PyBOP®	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphoniumhexafluorophosphate
HOBT	N-Hydroxybenzotriazole
AA	Amino acid

EXAMPLE 1

Preparation of a Construct containing a Photolytically Cleavable First Cleavage Site

A photocleavable construct was prepared following the reaction scheme shown in Scheme 1. In this example, the photolytic cleavage of a substituted nitrobenzyloxycarbonyl linker group attached to one amino group of an ethylene diamine chain liberates the free amino group so that it is capable of undergoing ionisation in a mass spectrometer thereby enabling a

characteristic molecular ion to be formed. In this example, the ethylene diamine connecting group has an isotopically labelled benzyl group attached to the other amino group, the effect of which is to provide a characteristic split peak in the mass spectrum of the cleavage product to assist identification.

The compound numbers used in the Experimental for Example 1 refer to the compound numbers in Scheme 1.

24

Scheme 1

* 1:1 ¹H₂:²H₂
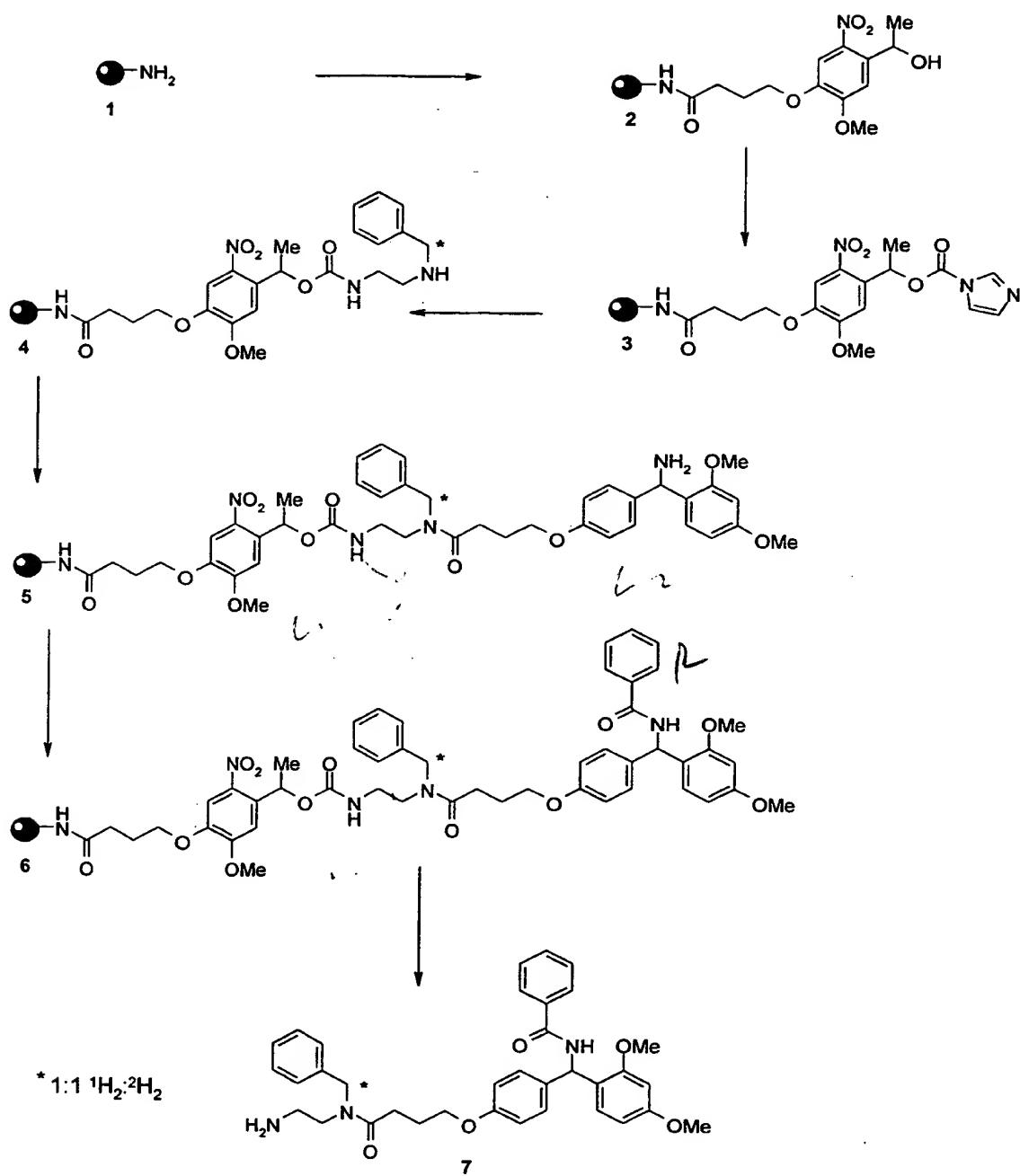
Experimental For Scheme 1

TentaGel NH$_2$ (Rapp Polymere) (Resin 1) (30 g, 12.0 mmol), having a bead diameter in the range 130 μm to 160μm and a loading of 0.4 mmol g^{-1}, was swelled with DMF and to this was added a solution of HOBT (9.3 g, 69.0 mmol), DIC (6.48 ml, 41.4 mmol) and 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy) butanoic acid (11.8 g, 39.4 mmol) in DMF (420 ml) and the mixture shaken for 16 h. The resin was then drained and washed with DMF followed by MeOH then DCM and finally MeOH. This was then dried *in vacuo* to give resin 2.

Resin 2 (3 g, 11.9 mmol) was treated with a solution of carbonyldiimidazole (19.3 g, 119 mmol) in DCM (400 ml) and the whole heated at 50 °C with slow stirring for 5 h. The resin was then drained and washed with DCM followed by Et$_2$O, DCM, Et$_2$O and dried *in vacuo* to give resin 3.

Resin 3 (15 g, 4.35 mmol) was swelled with DMF (200 ml) and to this was added a solution of 1-benzyl-1-*tert*-butoxycarbonyl-1,2-diaminoethane (17) (2.71 g, 10.9 mmol) and 1-(dideuterobenzyl)-1-*tert*-butoxycarbonyl-1,2-diaminoethane (16) (2.71 g, 10.9 mmol) in DMF (20 ml) and the whole heated at 50 °C with slow stirring for 16 h. The resin was drained and washed with DMF followed by Et$_2$O, DCM, and Et$_2$O. The resin was then dried *in vacuo*. The resin was then treated with a 1:4 solution of aq.TFA (95%) and DCM and shaken for 30 min. The resin was then drained and washed with DCM followed by Et$_2$O, DCM, Et$_2$O and the resin was dried *in vacuo*. The resin was then shaken with 10% solution of DIPEA in DMF for 1h and then drained, the washing was then repeated as above to give resin 4.

A solution of HATU (0.96 g, 2.52 mmol), DIPEA (0.89 ml, 5.04 mmol) and p-[(R,S)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxy-benzyl]-phenoxy-butanoic acid (1.42 g, 2.52 mmol) in DMF (30 ml) was added to Resin 4 (3 g, 0.84 mmol) and the whole shaken for 16 h. The resin was then drained and washed with DMF, and DCM. A solution of 20% piperidine in DMF (30 ml) was added to the resin and the whole shaken for 1h. The resin was then drained and washed with DMF, DCM followed by Et$_2$O, DCM and Et$_2$O and the resin was dried *in vacuo*.

Resin 5 (20 mg, 0.004 mmol) was treated with a solution of HATU (15 mg, 0.04 mmol), DIPEA (14 μl, 0.08 mmol) and benzoic acid (5 mg, 0.04 mmol) in DMF (1 ml) and the

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whole was shaken for 6 h. The resin was then drained and washed with DMF, DCM, Et₂O, DCM and Et₂O and dried *in vacuo* to give resin 6. Construct analysis of this resin by photocleavage followed by MS analysis is shown in figure 1 (see general cleavage conditions).

As can be seen from Figure 1, the presence of the sensitising amine group and peak splitter group in the analytical fragment cleaved from the resin to demonstrate a strong molecular ion with a characteristic doublet pattern.

General method of Construct Analysis of Resins via the photocleavable linker.

A small sample of the resin (ca. 0.5 mg) is suspended in DMSO (50 µl) and exposed to UV light for 30 min. The solution is then analysed by mass spectrometry. (Alternatively DMSO containing 0.2% hydrazine hydrate can be used as the solvent).

The mass spectra were obtained on a Finnegan MAT LCQ ion trap Mass Spectrometer operating in positive electrospray mode. Scan range 100 -1500 a.m.u. Scan cycle time 1.4 seconds; ion time 200 microseconds

EXAMPLE 2
Preparation of a Construct Containing a "Safety Catch" Thiopyrimidine Linker Group at the First Cleavage Site

A construct containing a thiopyrimidine "Safety Catch" linker at the first cleavage site was prepared according to the reaction scheme shown in Scheme 2 below. The construct numbers used in the following experimental description refer to the numbers in Scheme 2.

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Scheme 2

28
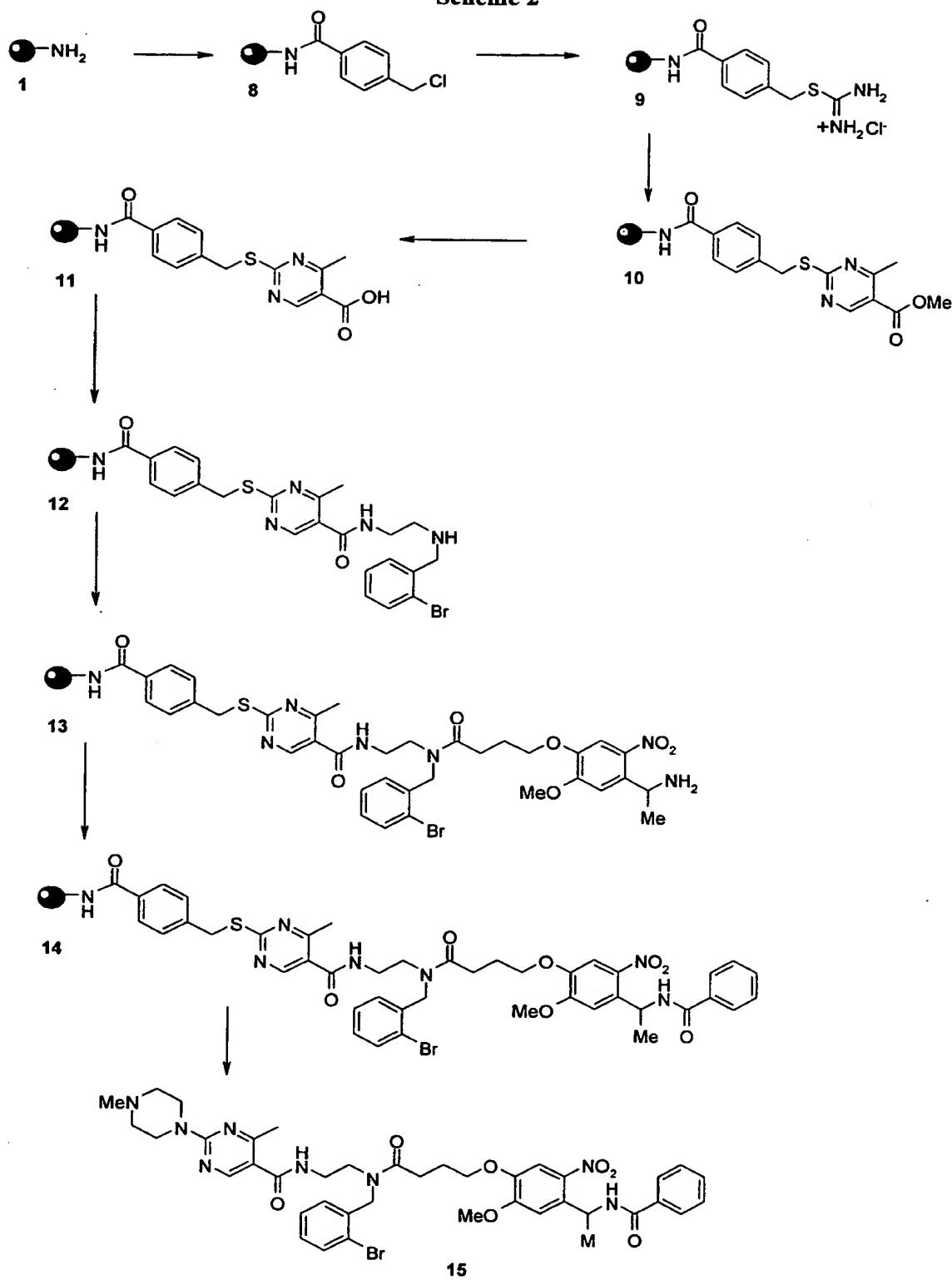
Experimental f r Scheme 2

A solution of 4-(chloromethyl)benzoic acid (0.27 g, 1.6 mmol), PyBOP® (0.83 g, 1.6 mmol) and DIPEA (0.56 ml, 3.2 mmol) in DMF (8 ml) was shaken for 20 min then this solution was added to TentaGel NH2 (Rapp Polymere) resin 1 (1 g, 0.32 mmol)), having a bead diameter in the range 130 μm to 160μm and a loading of 0.32 mmol g^{-1}, and the whole shaken for 5h. The resin was then drained and washed with DMF, DCM, Et_2O, DCM and finally Et_2O. The resin (8) was then dried *in vacuo*.

A slurry of thiourea (0.58 g, 7.7 mmol) in dioxane (4 ml) and ethanol (1 ml) was added to Resin 8 (1 g, 0.32 mmol) and the whole heated at 80 °C for 16h. The resin was drained and washed with DMF (x 5), DCM (x 5), Et_2O, DCM and finally Et_2O to give resin 9. The resin was then dried *in vacuo*.

A solution of methyl-2-(dimethylaminomethylene)-3-oxobutanoate (0.17 g, 0.96 mmol) and triethylamine (65 μl, 0.48 mmol) in DMF (10 ml) was added to resin 9 (1 g, 0.31 mmol) and the whole heated at 80 °C for 16h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin (10) was then dried *in vacuo*.

Resin 10 (0.5 g, 0.15 mmol) was then treated with a 1:1 mixture of THF and 2N aq.NaOH (4 ml) and shaken for 2h. The resin was then drained and washed with THF/H_2O, 10% AcOH/THF, THF/H_2O, THF, DMF, DCM, Et_2O, DCM, Et_2O and then dried *in vacuo* to give resin 11.

A solution of PyBOP® (24 mg, 0.045 mmol) and DIPEA (16 μl, 0.09 mmol) in DMF (1 ml) was added to resin 11 (50 mg, 0.015 mmol) followed by a solution of 1-*tert*-butoxycarbonyl-1-(*o*-bromobenzyl)-diaminoethane (18) (15 mg, 0.045 mmol) in DMF (1 ml) and the whole shaken for 3 days. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. A solution of 95% aq.TFA in DCM (20%, 1 ml) was then added to the resin and shaken for 2h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and then shak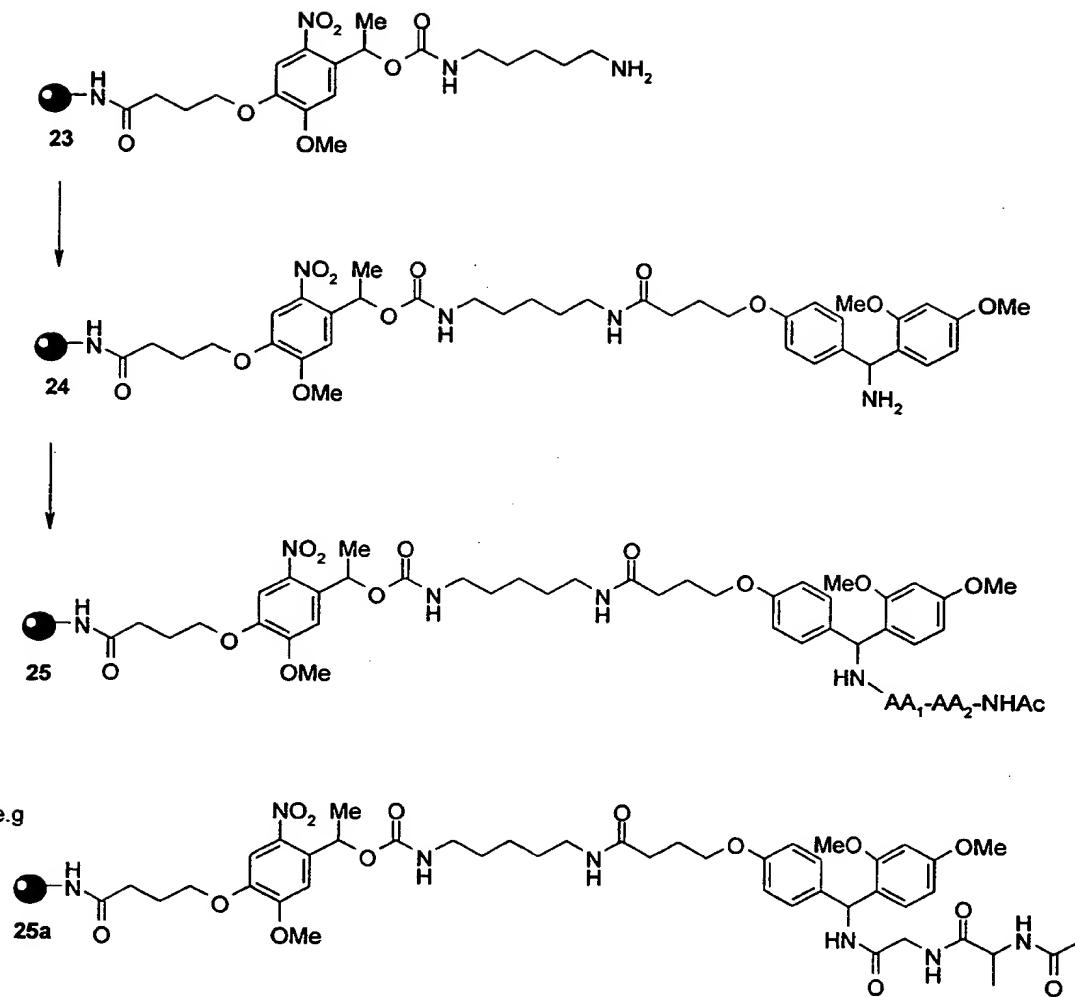en with 10% DIPEA in DMF for 10 min. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin (12) was then dried *in vacuo*.

A solution of 4-[4-(1-(Fmoc-amino)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (23 mg, 0.045 mmol), DIPEA (16 μl, 0.09 mmol) and HATU (17 mg, 0.045 mmol) in DMF (2 ml)

was added to resin **12** (50 mg, 0.015 mmol) and the whole shaken for 3h. The resin was drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then treated with 20% piperidine in DMF (3 ml) and the whole shaken for 1h. The resin (**13**) was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O and dried *in vacuo*.

A solution of benzoic acid (11 mg, 0.09 mmol), DIPEA (16 µl, 0.09 mmol) and HATU (17 mg, 0.045 mmol) in DMF (2 ml) was added to resin **13** and the whole shaken for 2h. The resin (**14**) was then drained and washed with DMF, DCM, Et_2O, DCM, and finally Et_2O. Construct analysis of this resin by pyrimidine cleavage conditions (described below) followed by MS analysis is shown in figure **2**.

General method of Construct Analysis of Resins via the Pyrimidine Linker.

A small sample of resin (ca. 0.5 mg) is treated with 0.01M m-chloroperbenzoic acid (0.5 ml) for 2h. The resin is then drained and washed with DCM, Et_2O and DCM. The resin is then treated with 0.02 M N-methylpiperazine in DMF (50 µl) is then added and shaken for 12h. The solution is then removed and analysed by mass spectrometry.

EXAMPLE 3
Preparation of Constructs Containing Different Peak Splitting Groups

A number of different mass spectrometric peak splitting groups have been prepared, and these are illustrated in Table 1 below.

30

R=	

Table 1

In Example 1, the peak splitting group was introduced by means of the mixture of deuterium labelled and unlabelled compounds **16/17** in Table 1 (see reference to resin **4**) whereas in Example 2, the peak splitting group was introduced by means of the bromo-benzyl compound **18** (see reference to resin **12**).

Resins containing alternative peak-splitting groups were prepared using the compounds **19/20** and **21/22** as described below.

Use of amines 19/20

A solution of 1-*tert*-butoxycarbonyl-1,2-diaminoethane (**20**) (110 mg, 0.68 mmol) and doubly ^{15}N labelled 1-*tert*-butoxycarbonyl-1,2-di(^{15}N)aminoethane (**19**) (112 mg, 0.68 mmol) in DMF was added to resin **3** (0.6 g, 0.18 mmol) and the whole heated at 50 °C for 3h. The resin was then drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin was then dried *in vacuo*.

Use of amines 21/22

A solution of 1-*tert*-butoxycarbonyl-1,5-diaminopentane (21) (295 mg, 1.45 mmol) and doubly ^{15}N labelled 1-*tert*-butoxycarbonyl-1,2-di(^{15}N)aminopentane) (22) (295 mg, 1.45 mmol)

5 in DMF was added to resin 3 (1.66 g, 0.58 mmol) and the whole heated at 50 °C for 4h. The resin was then drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. A solution of 95% aq.TFA in DCM (20%, 1 ml) was then added to the resin and shaken for 2h. The resin was drained and washed with DMF, DCM, Et$_2$O, DCM, and then shaken with 10% DIPEA in DMF for 10 min. The resin (23) was then dried *in vacuo*.

10

EXAMPLE 4
Preparation of Constructs Containing Dipeptide Substrates

In Examples 1 and 2, the substrate or target compound in each case is the model

15 compound benzamide. In the following Examples, constructs were prepared containing dipeptides, as the substrate, bound to either a Rink linker or a Wang linker as the second linker group. In each case, a photocleavable first linker group was used. The reaction schemes used to prepare the constructs are shown in Schemes 3 and 4 below.

32

Scheme 3

Scheme 4

5

34
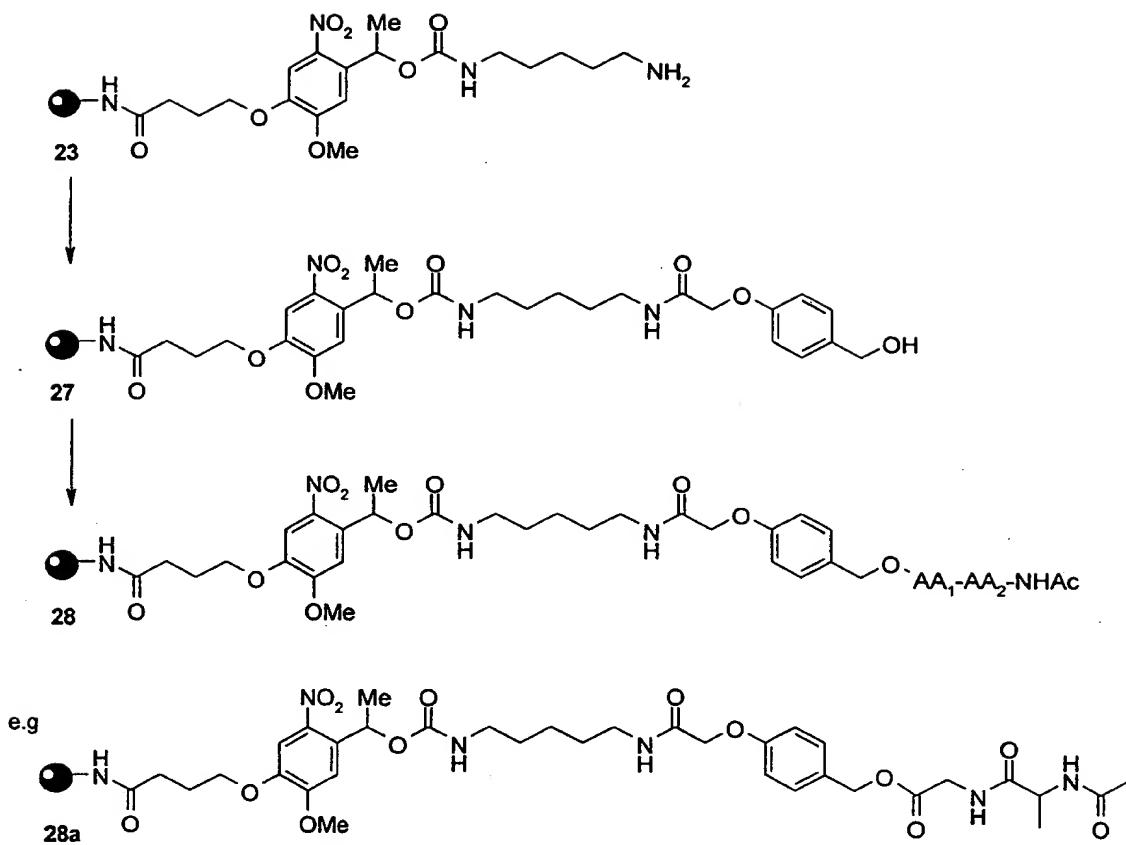
Experimental for Amin acid examples

Preparation of Rink resin 24

5

A solution of p-[(R,S)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxy-benzyl]-phenoxybutanoic acid (240 mg, 0.42 mmol), DIC (65 μl, 0.42 mmol) and HOBT (57 mg, 0.42 mmol) in DMF (1 ml) was added to resin 23 (100 mg, 0.042 mmol) and the whole shaken for 4h. The resin was then drained and washed with DMF, DCM, Et$_2$O, DCM, and

10   finally Et$_2$O. The resin was then treated with 20% piperidine in DMF (2 ml) and shaken for 30 min. The resin (24) was then drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin was then dried *in vacuo*.

Preparation of Wang resin 27.

15

A solution of 4-formylphenoxyacetic acid (23 mg, 0.13 mmol), DIC (20 μl, 0.13 mmol) and HOBT (17 mg, 0.13 mmol) in DMF (1 ml) was added to resin 23 (100 mg, 0.042 mmol) and the whole shaken for 4h. The resin was then drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin was then dried *in vacuo*. The resin was then treated with a

20   solution of tetrabutylammonium borohydride (50 mg, 0.2 mmol) in DCM and shaken for 16h. The resin (27) was then drained and washed with DCM, DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin was then dried *in vacuo*.

Preparation of acylated dipeptide sequence on resin.

25

	RINK (25)			WANG (28)	
	AA1	AA2		AA1	AA2
Example 25a	Gly	Ala	Example 28a	Gly	Ala
Example 25b	Val	Gly	Example 28b	Val	Gly
Example 25c	Ala	Val	Example 28c	Ala	Val

Table 2: AA1 and AA2 on Rink and Wang linkers

The resins **25** and **28** were prepared by reacting the resins **24** and **27** respectively with the desired Fmoc protected amino acids followed by deprotection and then repeated coupling to the second aminoacid and deprotection (see general method). The terminal amino groups were then acylated using acetic anhydride (see method below).

Construct analysis of these resins by photocleavage followed by MS analysis gave the mass spectra shown in the figures according to table 3.

Analysed Resin	Figure
25a	3
25b	4
25c	5
28a	6
28b	7
28c	8

Table 3: Mass spectra after construct analysis

General/Typical Method f r coupling the amino acids to the linkers

A solution of the Fmoc protected amino acid (0.14 mmol, 10 Eq), DIC (22 µl, 0.14 mmol, 10 Eq) and HOBT (19 mg, 0.14 mmol, 10 Eq) in DMF (1ml) was added to required resins 24 and 27 (30 mg, ca. 0.014 mmol). The whole was then shaken for 5h. The resin was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O. The Fmoc protecting groups were then be removed by the treatment of the resins with 20% piperidine solution in DMF (2 ml) and shaking for 30 min. The resin was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O.The resin was then dried *in vacuo*.

Acetylation of the dipeptides

A solution of acetic anhydride (15 ml, 0,14 mmol) in DCM (1 ml) was added to the resins (30 mg, ca. 0.014 mmol) followed by DMAP (1 mg, 0.008 mmol) and the whole shaken for 2h. The resin was then drained and washed with DCM, DMF, DCM, Et_2O, DCM, and finally Et_2O. The resin was then dried *in vacuo*.

As can be seen from the above examples, the constructs of the invention enable small molecules formed by solid phase methods to be analysed readily and easily by mass spectrometry. By providing an ionisable group as a mass spectral sensitiser, and a peak splitting isotope, the result in each case has been a readily interpreted mass spectrum in which the molecular ion appears as a characteristic doublet, and in which peaks due to fragments or extraneous materials are negligible.

EXAMPLE 5
Preparation of a Construct Containing a Sulphonamide Linker at the First Cleavage Site

A construct containing benzoic acid as a model substrate linked via an acid labile "Rink" type linker to an ethylene diamine mass spectral peak splitting group and in turn via a sulphonamido linker to a resin was prepared as shown in Scheme 5 below. In this Example, cleavage of the sulphonamide with mercaptoethanol in the presence of strong base releases an analytical fragment having a mass spectrometric sensitising amino group

Scheme 5

5

3a R=²H, 3b R=¹H

4a R=²H, 4b R=¹H
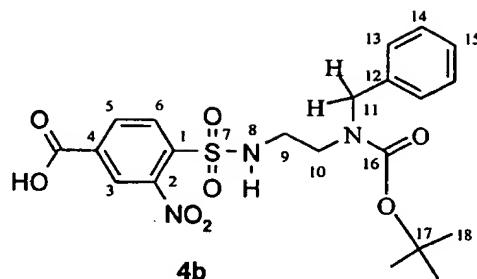
5

6

7

8

38

Preparation of Compound 3a

3a

5
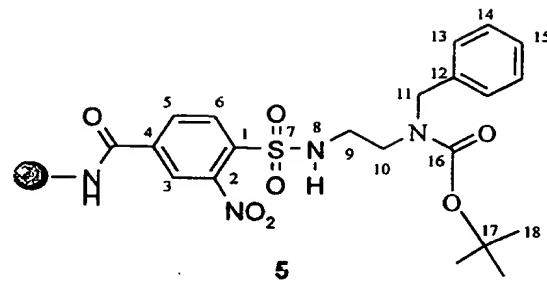
To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride 1 (1.10 g, 3.90 mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2a** (1.00 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 2 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished *the sulfonamide 3a* (1.53 g, 79%) as white flakes; mp 129-130°C; R_f 0.31 [MTBE:hexane (1:1)]; (Found: C, 53.2; H, 5.6; N, 8.5; S, 6.4. $C_{22}H_{25}D_2N_3O_8S$ requires C, 53.3; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.40 (9H, s, H-18), 3.05 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 3.9 (3H, s, O-Me), 7.10-7.35 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.35 (1H, d, *J* 8, H-5), 8.40 (1H, m, H-8), 8.45 (1H, s, H-3),; δ_C (DMSO-d^6) 27.8 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 53.0 (O-Me), 79.1 (C-17), 124.8 (C-13), 127.0 (C-14 and 15), 128.3 (C-6), 130.0 (C-13), 132.9 (C-5), 134.2 (C-12), 136.4 (C-1), 147.5 (C-4), 154.7 (C-2), 163.5 (C-16 and C=O); MS (Electrospray +ve) m/z 496 (MH)$^+$; HPLC, R_t 6.07 min.

20

Preparati n of Compound 4a

4a

The ester **3a** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to *the acid 4a* (0.34 g, 88%) as a white solid; mp 143-145°C; R_F 0.58 [MTBE:hexane (1:1)]; δ_H (DMSO-d^6) 1.29 (9H, s, H-18), 3.02 (2H, m, H-9), 3.20 (2H, dt, J 3 and 13, H-10), 7.10-7.40 (5H, m, H-13, 14 and 15), 8.06 (1H, d, J 8, H-6), 8.28 (1H, s, H-3), 8.30 (1H, d, J 8, H-3); δ_C (DMSO-d^6) 27.9 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.5 (C-13), 127.1 (C-14 and 15), 128.4 (C-6), 129.5 (C-13), 132.5 (C-5), 139.2 (C-12), 147.3 (C-1), 155.1 (C-4), 164.8 (C-16 and C=O); (Found: MH^+, 482.156364 $C_{21}H_{23}D_2N_3O_8S$ requires MH+, 482.156615); HPLC, R_t 5.65 min.

40

Preparati n of Compound 3b

3b

5

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride **1** (1.10 g, 3.90

10   mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C

the amine **2b** (0.95 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction

mixture was stirred for a further 3 h at room temperature then concentrated *in vacuo* to give a

yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by

recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished *the sulfonamide 3b* (1.51 g,

15   79%) as white flakes; mp 131-132°C; R_f 0.28 [MTBE:hexane (1:1)]; (Found: C, 53.7; H, 5.4;

N, 8.4; S, 6.5. $C_{22}H_{27}N_3O_8S$ requires C, 53.5; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.38 (9H,

s, H-18), 3.10 (2H, t, J 8, H-9), 3.24 (2H, t, J 8, H-10), 3.95 (3H, s, O-Me), 4.32 (2H, s, H11),

7.13-7.32 (5H, m, H-13, 14 and 15), 8.09 (1H, d, J 8, H-6), 8.29 (1H, s, H-3), 8.32 (1H, d, J 8,

H-3); δ_C (DMSO-d^6) 28.3 (C-18), 40.2 (C-9 and 10), 46.2 (C-11), 53.2 (O-Me), 79.3 (C-17),

20   125.1 (C-13), 127.2 (C-14 and 15), 128.6 (C-6), 130.3 (C-13), 133.1 (C-5), 134.4 (C-12), 136.6

(C-1), 147.6 (C-4), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 492 (M-H)⁻; HPLC, R_t

5.99 min.

25

Preparation of Compound 4b

5

4b

The ester **3b** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous

10   sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The
reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then
acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white
precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts
were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to *the acid 4b*

15   (0.36 g, 93%) as a white solid; mp 123.7-124.8°C; R_F 0.59 [MTBE:hexane (1:1)]; δ_H (DMSO-
d^6) 1.42 (9H, s, H-18), 3.12 (2H, m, H-9), 3.22 (2H, m, H-10), 4.38 (2H, s, H11), 7.18-7.42
(5H, m, H-13, 14 and 15), 8.11 (1H, d, J 8, H-6), 8.39 (1H, d, J 8, H-3); 8.45 (1H, s, H-3); δ_C
(DMSO-d^6) 27.8 (C-18), 40.1 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.8 (C-13), 126.9 (C-
14 and 15), 128.4 (C-6), 129.9 (C-13), 132.9 (C-5), 135.8 (C-12), 137.9 (C-1), 147.5 (C-4),

20   153.9 (C-2), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 478 (M-H)⁻; HPLC, R_t 5.56
min.

Preparation of Compound 5

5

To a suspension of amine resin (Argogel-NH$_2$) (0.37 g, 0.15 mmol)), having an average bead diameter of 150 μm and a loading of 0.4 mmol g^{-1}, in DMF (4 ml) and dichloromethane (2 ml) was added the benzoic acid (4a) (0.22 g, 0.45 mmol), and the benzoic acid (4b) (0.22 g, 0.45 mmol), followed by PyBOP (0.47 g, 0.9 mmol) and HOBT (012. g, 0.90 mmol) followed, 4 min. later by Hünig's base (0.21 g, 1.80 mmol). The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the *sulfonamide resin 5* (0.43 g, 97%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative.

43

Preparation of Compound 6

D/D : H/H (50:50)

6
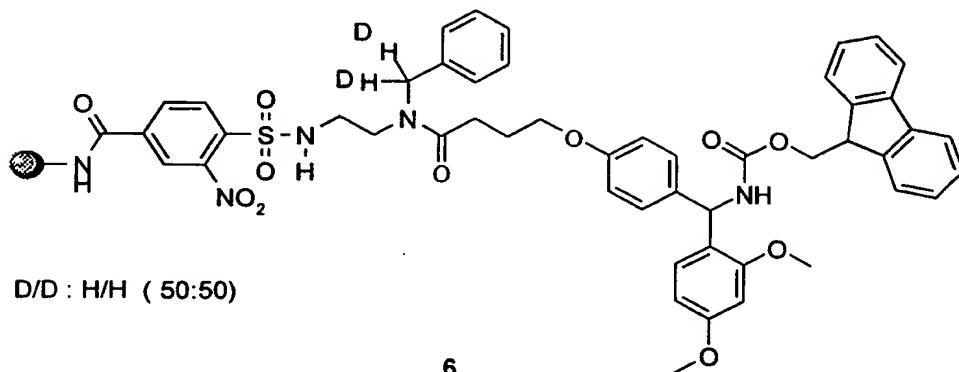
The protected resin (0.39 g, 0.13 mmol) was suspended in dichloromethane (2 ml) and treated with trifluoroacetic acid (2 x 4 ml) for 5 min. Solvents were removed by filtration and the resin washed with 10% DIPEA solution in DMF (3 x 5 ml), dichloromethane (6 x 5 ml), DMF (6 x 5 ml), dichloromethane (6 x 5 ml), ether (2 x 5 ml). The amine resin was then suspended in DMF (4 ml) and dichloromethane (2 ml) the Rink acid (0.51 g, 0.90 mmol) was added, followed by PyBOP (0.47 g, 0.9 mmol) and HOBT (012. g, 0.90 mmol). After 4 min. Hünig's base (0.21 g, 1.80 mmol) was added. The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the *sulfonamide resin 6* (0.43 g, 100%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative; Resin loading 98% as estimated by quantitative Fmoc group cleavage.

44

Preparation of Compound 7

D/D : H/H (50:50)

7
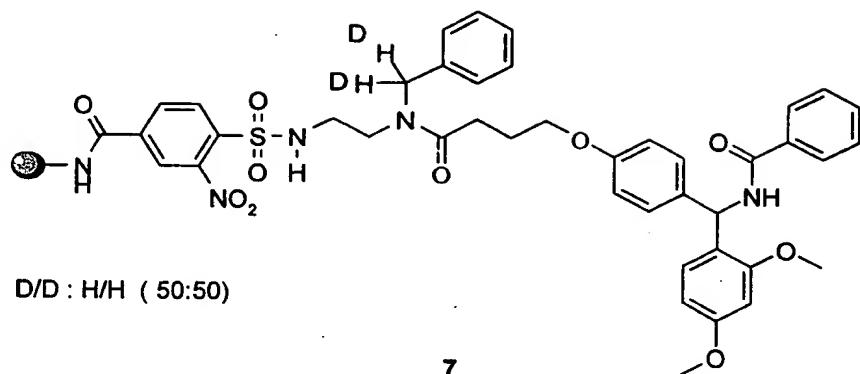
Resin **(6)** (0.20 g, 0.06 mmol) was treated with 20% Piperidine in DMF solution (2 x 3 ml) for 10 min. Solvents were removed by filtration and the resin washed with DMF (6 x 3 ml), dichloromethane (6 x 3 ml), ether (2 x 3 ml). The resin was suspended in DMF (1 ml) and dichloromethane (1 ml) and benzoic acid (12 mg, 0.01 mmol) was added, followed by PyBOP (52 mg, 0.1 mmol) and HOBT (13 mg, 0.1 mmol). After 4 min. Hünig's base (26 mg, 0.2 mmol) was added. The reaction was agitated with nitrogen for 3 h and the resin then washed with dichloromethane (6 x 1 ml), DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml) and dried *in vacuo* to give the *sulfonamide resin 7* (51 mg, 100%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative;

Preparation of Compound 8

D/D : H/H (50:50)

8
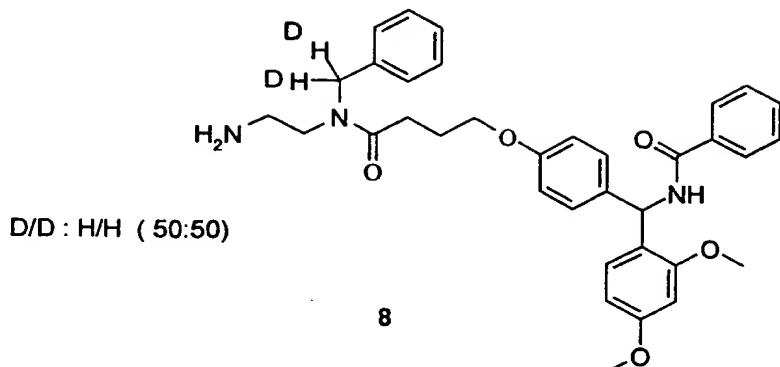
A small sample of resin (7) (ca. 0.5 mg) was treated with deprotecting solution A (0.5 ml) for 1 hr. The resin was filtered and the resulting solution was then analysed by electrospray mass spectrometry; MS (Electrospray -ve) m/z 582/584 (M-H)⁻; HPLC, R_t 4.49 min.

Solution A: Mercaptoethanol (0.23 ml, 3.0 mmol) and DBU (0.67 ml, 4.5 mmol) in MeCN (3 ml).

EXAMPLE 5

Preparation of a Construct Containing a dde-linker at the first Cleavage site

A construct containing a benzoic acid model substrate linked via a "Rink" type linker to an ethylene diamine mass spectral peak splitter and thence via a "dde" linker to a resin was prepared according to the reaction scheme set out in Scheme 6 below. Cleavage of the construct at the first cleavage site defined by the dde group using hydrazine generates an analytical fragment having a free amino group as a mass spectrometric sensitising group.

Scheme 6

R = 1:1 ¹H:²H
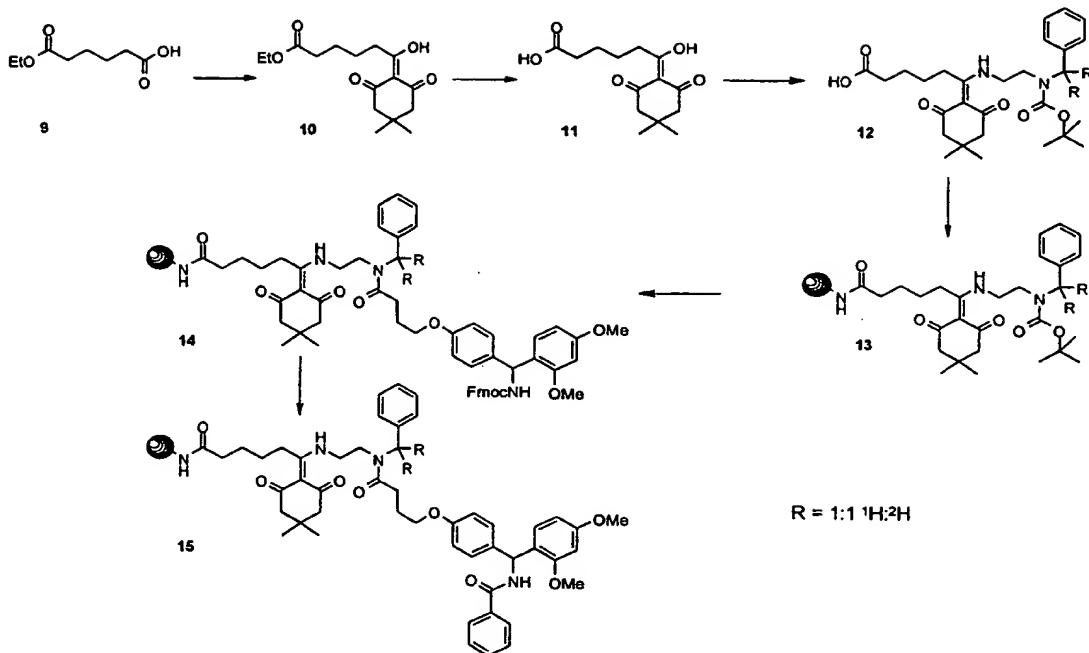
5

General

HPLC was run on a Hewlett Packard 1050 instrument using a C_{18} reverse phase column (Supelco, Supelcosil ABZ+, 3.3 mm, 4.6 mm ϕ). Method A: 10 to 95% solvent B gradient (1 ml/min) as the mobile phase. [Solvent A: 1% TFA in water; solvent B; 0.5% TFA in

10   MeCN:water (10:1), 8 min gradient time], or method B: C_{18} reverse phase column (Dynamax 60A, 25 mm, 6 mm ϕ) with 10 to 95% solvent B gradient (1 ml/min) as the mobile phase. [Solvent A: 1% TFA in water; Solvent B; 0.5% TFA in MeCN:water (10:1), 10 min gradient time]. Melting points were performed on a Mettler FP5 automatic melting point apparatus in open tubes heating from 50°C at 2°C min⁻¹ and are uncorrected.

Preparation of Compound 10
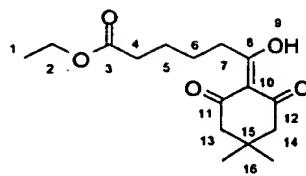
4-Dimethylaminopyridine (4.03 g, 33 mmol) was added to a solution of adipic acid monoethylester (5.33 ml, 30 mmol) in dimethylformamide (50 ml) followed by diisopropylcarbodiimide (5.17 ml, 33 mmol). After 25 min, a solution of dimedone (4.63 g, 33 mmol) in dimethylformamide (25 ml) was added and stirring continued for 48 h. The reaction mixture was diluted with ethyl acetate (300 ml) and washed with 0.5M KHSO$_4$ (2 x 100 ml), water (100 ml) and saturated brine (50 ml), then dried (MgSO$_4$), filtered and evaporated. The resulting slurry was triturated with ethyl acetate (250 ml), filtered and the residue washed with ethyl acetate. Evaporation of the combined filtrates gave a yellow gum which was purified by solid phase extraction (1.5" x 4" column, eluant: dichloromethane) to give *compound 10* as a yellow oil (7.43 g, 84%). δ_H (CDCl$_3$, 400MHz): 4.04 (q, 2H, H$_2$, 7 Hz), 2.97 (t, 2H, H$_4$, 7.5 Hz), 2.46 (s, 2H, H$_{13}$), 2.28 (s, 2H, H$_{14}$), 2.26 (d, 2H, H$_7$, 7.5 Hz), 1.60 (m, 4H, H$_{5,6}$), 1.00 (s, 6H, H$_{16}$). δ_C (CDCl$_3$, 400 MHz): 205.15 (C-12), 197.58 (C-11), 195.08 (C-3), 173.47 (C-8), 112.02 (C-10), 60.27 (C-2), 52.67 (C-4), 46.80 (C-13), 40.04 (C-14), 34.15 (C-7), 30.72 (C-15), 28.23 (C-16), 24.64 (C-5), 24.01 (C-6), 14.31 (C-1). v_{max} (cm^{-1}): 2960 (OH), 1734 (C=O ester), 1665 (C=O ketone), 1564, 1445, 1143. MS: (electrospray +ve) m/z 297 (MH)$^+$ (electrospray - ve), 295 (M-H)$^-$. HPLC method A: 5.31 min, 97%. 254nm.
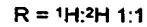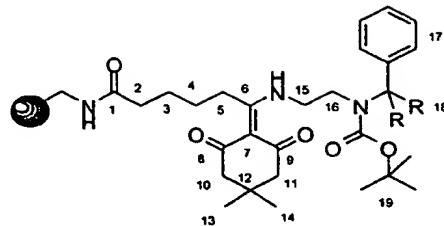
Preparation of Compound 11
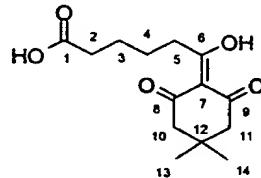
2M sodium hydroxide solution (2 ml, 4 mmol) was added to a solution of compound 10 in tetrahydrofuran (5 ml). After 3 h, the reaction mixture was poured onto 10% aq. hydrochloric acid (50 ml) and extracted with ethyl acetate (2 x 50 ml). The combined organic phases were dried (MgSO$_4$), filtered and evaporated to give *compound 11* as an off white solid

(498.5 mg, 95%). δ_H (CDCl$_3$, 400MHz): 2.99 (dd, 2H, H$_2$, 5 Hz), 2.47 (s, 2H, H$_{10}$), 2.34 (dd, 2H, H$_5$, 5Hz), 2.29 (s, 2H, H$_{11}$), 1.63 (m, 4H, H$_{3,4}$), 1.00 (s, 6H, H$_{13,14}$). δ_C (CDCl$_3$, 400 MHz): 205.15 (C-9), 197.58 (C-8), 195.08 (C-1), 173.47 (C-6), 112.02 (C-7), 52.96 (C-2), 47.11 (C-10), 40.40 (C-11), 33.92 (C-5), 30.72 (C-12), 28.23 (C-13,14), 24.64 (C-3), 24.01 (C-4). ν_{max} (cm^{-1}): 3030 (acid OH), 1702 (acid C=O), 1646 (ketone C=O). Melting point: 58.2°C. MS: (electrospray +ve) m/z 269 (MH)$^+$ (electrospray -ve), 267 (M-H)$^-$. HPLC method A: 4.15 min, 97%. 254nm.

Preparation of Compound 12

R = ^{1}H:^{2}H 1:1
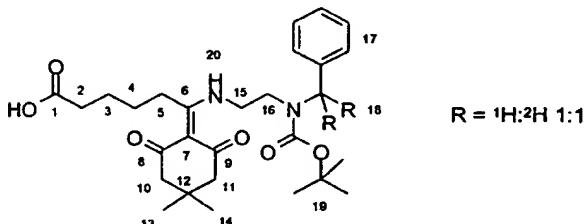
A solution of N-(*tert*-butoxycarbonyl)-N-benzyldiaminoethane (187.3 mg, 0.75 mmol) and N-(*tert*-butoxycarbonyl)-N-(α-dideutero)benzyldiaminoethane (189.3 mg, 0.75 mmol) in dichloromethane (3 ml) followed by diisopropylethylamine (348.4 μl, 2 mmol) was added to a solution of compound 11 (268.4 mg, 1 mmol) in dichloromethane (2 ml). After stirring for 20 h, the reaction mixture was evaporated, redissolved in ethyl acetate (25 ml) and washed with 10% citric acid solution (3 x 10 ml), water (10 ml) and saturated brine (10 ml). The organic phase was dried (MgSO$_4$), filtered and evaporated then purified by flash chromatography (1 to 3% methanol in chloroform) to give *compound 12* as a yellow gum (474.5mg, 95%). δ_H (CDCl$_3$, 400MHz): 13.50 (bs, 1H, H$_{20}$), 7.30 (m, 5H, H$_{17}$), 4.45 (bd, 1H, H$_{18}$), 3.47 (bd, 4H, H$_{15,16}$), 2.90 (m, 2H, H$_2$), 2.40 (t, 2H, H$_5$, 7.4Hz), 2.30 (s, 4H, H$_{10,11}$), 1.75 (m, 2H, H$_4$), 1.45 (m, 11H, H$_{3,19}$). ν_{max} (cm^{-1}): 3030 (acid OH), 1730 (C=O, Boc, acid), 1690 (ketone C=O), 1570 (conj. enamine). MS: (electrospray +ve) m/z 503, 501 (MH)$^+$ (electrospray -ve), 502, 499 (M-H)$^-$. HPLC method A: 5.35 min, >97%. 230, 254nm.

49

Preparation of C mpound 13

R = ¹H:²H 1:1

To a solution of compound **12** (310 mg, 0.62 mmol) in dichloromethane (6 ml) was added 1-hydroxybenzotriazole (110.8 mg, 0.82 mmol) and PyBOP (426.6 mg, 0.82 mmol).

5   After 5 min, the solution was added to a suspension of aminomethyl Argogel® (500 mg, 0.205 mmol, 0.41 mmol/g loading) having an average bead size of 150μm, in dichloromethane (1 ml). The reaction mixture was agitated for 5 min then diisopropylethylamine (285.7μl, 1.64 mmol) was added and agitation continued for 16 h. The resin was washed with DMF (5 x) and DCM (5 x) then dried *in vacuo* to give *resin 13* (573.5 mg). The resin gave a negative bromophenol

10 blue test.

Preparation of Compound 14

R = 1:1 ¹H:²H   FmocNH   OMe
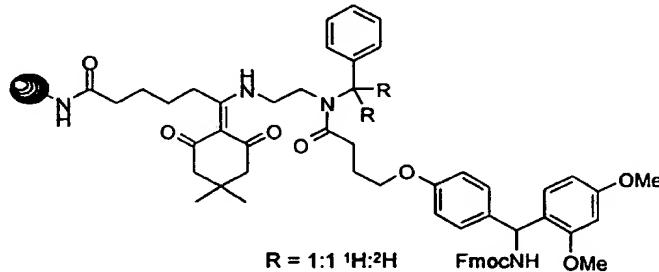
15 Resin **13** (475.5 mg) was swollen with dichloromethane then treated with a solution of phenol (60 mg) in dichloromethane (1ml) followed by trifluoroacetic acid (3ml). The reaction mixture was agitated for 10 min then drained and washed with dichloromethane (2 x). The reaction was repeated then the resin washed with dichloromethane (1 x), 20% diisopropylethylamine in dimethylformamide (3 x), dimethylformamide (5 x) and

20 dichloromethane (5 x) to give the amino resin intermediate which gave a positive bromophenol blue. The resin was suspended in dichloromethane (2 ml) and treated with a preformed solution of the C-4 Rink acid (351.5 mg, 0.62 mmol), 1-hydroxybenzotriazole (110.8 mg, 0.82 mmol) and PyBOP (426.6 mg, 0.82 mmol) in dimethylformamide (2 ml). After 5 min,

diisopropylethylamine (285.7µl, 1.64 mmol) was added and the reaction mixture agitated for 3 h after which the resin gave a negative bromophenol blue test. The resin was washed with DMF (5 x) and DCM (5 x) then dried *in vacuo* to give *resin 14* (500.9 mg).

5   A small sample of resin 14 (ca. 0.5 mg) was treated with 2% hydrazine in dimethyl formamide for 30 minutes. The solution was then analysed by electrospray mass spectrometry to give an ion 700/702.

Preparation of Compound 15

R = 1:1 ^{1}H:^{2}H
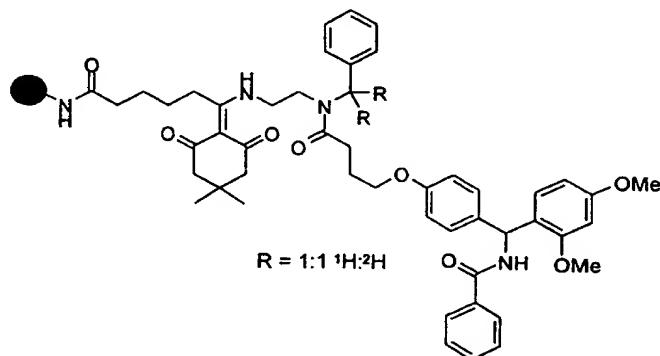
10   Resin 14 (65 mg) was treated with 20% piperidine in dimethylformamide (2 x 3 ml x 10 min) then washed with dimethylformamide (5 x) and dichloromethane (5 x). The resin gave a positive Kaiser test and was suspended in dichloromethane (1 ml) then treated with a preformed solution of benzoic acid (127 mg, 1.04 mmol), 1-hydroxybenzotriazole (140 mg, 15   1.04 mmol) and PyBOP (541 mg, 1.04 mmol) in dimethylformamide (2 ml). After 5 min, diisopropylethylamine (541 mgµl, 2.08 mmol) was added and the reaction mixture agitated for 72 h after which the resin gave a negative Kaiser test. The resin was washed with DMF (5 x) and DCM (5 x) then dried *in vacuo* to give *resin 15* (60 mg).

20   A small sample of the resin (ca. 0.5mg) was treated with 2% hydrazine in dimethylformamide for 30 minutes. The solution was then analysed by electrospray mass spectrometry to an ion 582/584.

EXAMPLE 6
Preparation of a 64-Membered Library By a 4x4x4 Combinatorial Synthesis

The usefulness of the constructs of the invention in creating combinatorial libraries

5   was demonstrated by preparing a 64 membered library of constructs containing different diamide substrates using a 4x4x4 combinatorial synthetic procedure. In order to provide a means of determining the synthesis history of the beads, and to enable sorting and mixing of the beads to be positively controlled (rather than being determined merely by simple statistical distribution) so that all 64 members of the library were formed, quantities of the

10   resin beads were loaded into 64 radio frequency labelled Irori MicroKan ™ containers. The MicroKan ™ containers, available from Irori, of La Jolla, California, USA, are polypropylene containers having a polypropylene mesh side wall, a resin capacity of up to 30 mg and an internal volume of 330 µl. The use of the MicroKan ™ containers provides the benefits, in terms of numbers of compounds synthesised, of a split and pool

15   combinatorial synthesis, but avoids the need to build up coding constructs on the resin beads since the MicroKan ™ containers are provided with radio-frequency tags which enable each container to be identified and therefore monitored through the reaction sequence. Consequently, since each container contains a unique substrate at the end of the reaction sequence, and the synthesis history of the container has been tracked, the identity

20   of the substrate in each container can be determined, assuming of course that each reaction step has proceeded according to plan.

The diamide substrates were built up on a scaffold containing a thiopyrimidine safety catch linker at the first cleavage site, a diamino ethane connecting group carrying an

25   N-benzyl peak splitting group, and a photolabile linker at the second cleavage site joining the substrate to the diaminoethane connecting group.

An intermediate construct containing the N-benzyl diamino ethane peak splitter group and photolabile pyrimidine linker group was prepared by the reaction sequence

30   shown in Scheme 7 below, the subsequent synthetic steps and preparation of the combinatorial library being shown in Scheme 8. The experimental details for each scheme are set out below and the compound or construct numbers used in the experimentals refer to the construct/compound numbers in the two schemes.

52

Scheme 7

5
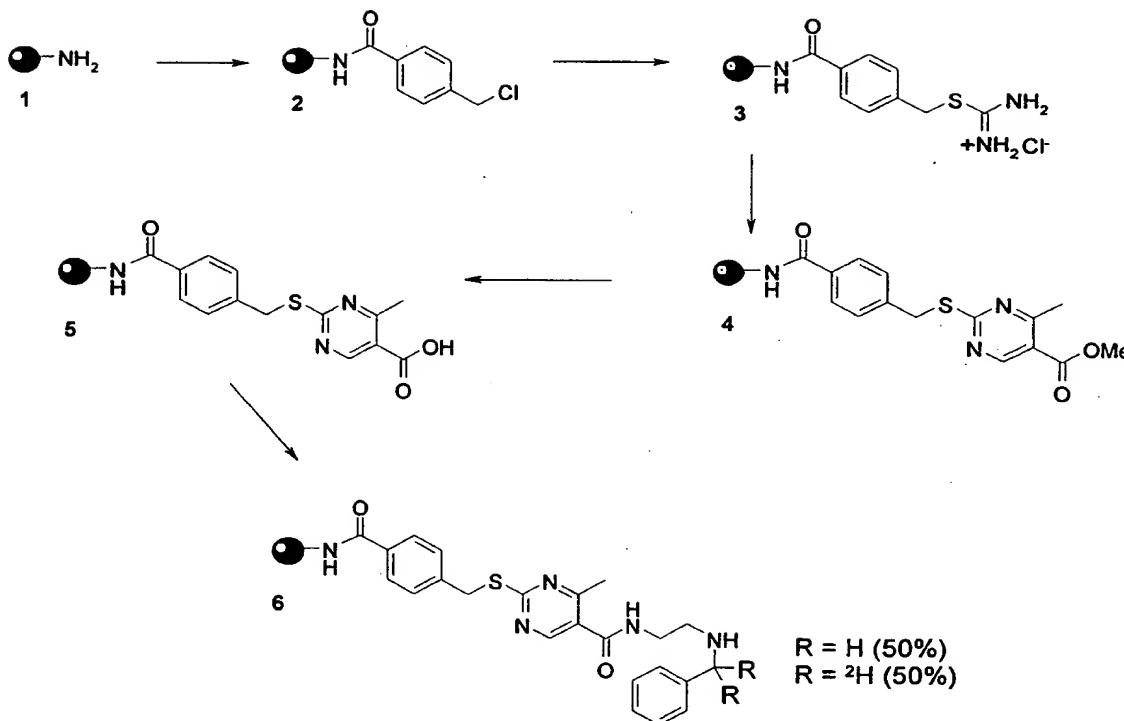
10   **Experimental for pyrimidine construct**

A solution of 4-(chloromethyl)benzoic acid (3.91 g, 23 mmol), HATU (8.74 g, 23 mmol) and DIPEA (7.95 ml, 46 mmol) in DMF (40 ml) was shaken for 20 min then this solution was added to Argogel resin **1** (1 g, 0.46 mmol)), having an average bead diameter

15   of 150 μm and a loading of 0.46 mmol g^{-1}, and the whole was shaken for 5h. The resin was then drained and washed with DMF, DCM, Et$_2$O, DCM and finally Et$_2$O. The resin (**2**) was then dried *in vacuo*.

A slurry of thiourea (8.4 g, 110 mmol) in dioxane (80 ml) and ethanol (20 ml) was

20   added to Resin **2** and the whole heated at 80 °C for 16h. The resin was drained and washed with DMF (x 5), DCM (x 5), Et$_2$O, DCM and finally Et$_2$O to give resin **3**. The resin was

then dried *in vacuo*.

A solution of methyl-2-(dimethylaminomethylene)-3-oxobutanoate (2.4 g, 13.8 mmol) and triethylamine (0.96 ml, 6.9 mmol) in DMF (100 ml) was added to resin **3** and
5   the whole heated at 80 °C for 16h. The resin was drained hot and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin (**4**) was then dried *in vacuo*. Resin **4** was then treated with a 1:1 mixture of THF and 2N aq.NaOH (100 ml) and shaken for 2h. The resin was then drained and washed with THF/H$_2$O, 10% AcOH/THF, THF/H$_2$O, THF, DMF, DCM, Et$_2$O, DCM, Et$_2$O and then dried *in vacuo* to give resin **5**.
10

A solution of PyBOP® (3.6 g, 6.9 mmol) and DIPEA (2.4 ml, 25 mmol) in DMF (30 ml) was added to resin **5** (5 g, 2.3 mmol) followed by a solution of 1-benzyl-1-*tert*-butoxycarbonyl-1,2-diaminoethane (0.86 g, 3.5 mmol) and 1-(dideuterobenzyl)-1-*tert*-butoxycarbonyl-1,2-diaminoethane (0.86 g, 3.5 mmol) in DMF (1 ml) and the whole
15   shaken for 4 h. The resin was drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. A solution of 20% aq.TFA in DCM (20%, 30 ml) was then added to the resin and shaken for 2h. The resin was drained and washed with DMF, DCM, Et$_2$O, DCM, and then shaken with 10% DIPEA in DMF for 10 min. The resin was drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin (**6**) was then dried *in vacuo*.

54
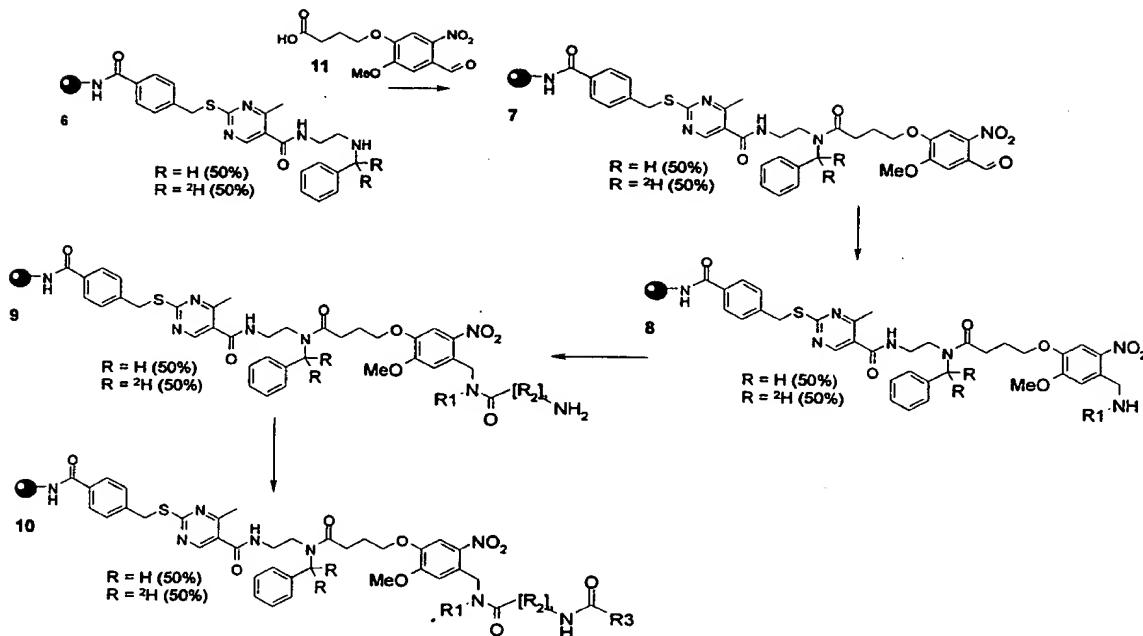
Scheme 8

5   **Experimental for Scheme 8**

The photolinker acid **11** (0.416 g, 1.47mmol), DIPEA (0.51 ml, 2.94 mmol) and PyBOP (0.765 g, 1.47 mmol) were dissolved in DMF (10ml) and left to stand for 10 mins. This was then added to resin 6 (0.7 g, 0.294 mmol) and shaken for 4 h. The resin was drained and washed with DMF, DCM, Et$_2$O, DCM, and then shaken with 10% DIPEA in
10   DMF for 10 min. The resin was drained and washed with DMF, DCM, Et$_2$O, DCM, and finally Et$_2$O. The resin (7) was then dried *in vacuo*.

General Procedure for the Reductive Amination Step

To a vessel containing 16 Irori MicroKan™ containers (each containing 10 mg,
15   0.004 mmol resin 7) in DCM (30 ml) was added a pre-mixed solution of the amine (for amounts see the table below) and acetic acid (46 μl, 0.768 mmol) in DCM (2 ml) and shaken for 20 min. To the vessel was then added a mixture of tetramethylammonium borohydride (0.404 mg, 1.54 mmol) and acetic acid (46 μl, 0.768 mmol) in DCM (5 ml) and the vessel shaken for 16 h. The vessels were then drained and the MicroKan (TM)
20   containers washed with DCM, DMF, DCM, and Et$_2$O (each 3 times).

R1		
A	[structure: 4-methylbenzylamine with H₃C and NH₂]	46 mg
B	[structure: tert-butyl ester of glycine, H₃C, CH₃, O, NH₂, ClH]	64 mg (67 µl DIPEA also added)
C	[structure: Boc-protected diaminoalkane, H₃C, CH₃, O, N-H, NH₂]	78 mg
D	[structure: H₃C—CH₂—CH₂—NH₂]	23 mg

General Procedure for the Acylation Step with amino acids

To each of 4 vessels containing 16 Irori MicroKan™ containers (each can containing 10 mg, 0.004 mmol resin 8) in DMF (30 ml) was added a solution of PyBOP (333 mg, 0.64 mmol), DIPEA (224 µl, 1.28 mmol) and one of the amino acids (see the table below for amounts) in DMF (5 ml) and the mixture shaken for 16 h. The vessels were then drained and the MicroKan™ containers washed with DMF, DCM, DMF, DCM, and Et2O (each 3 times). To a vessel containing all 64 MicroKan™ containers was then added a solution of piperidine (20%) in DMF and shaken for 3 h. The vessels were then drained and the MicroKan™ containers washed with DMF, DCM, DMF, DCM, and Et2O (each 3 times).
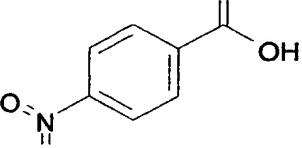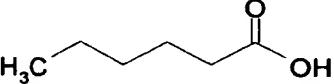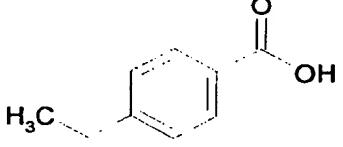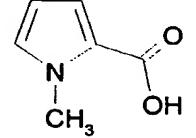
R2		
E	[structure: Fmoc-glycine, O, H-N, O, OH, fluorenyl]	190 mg
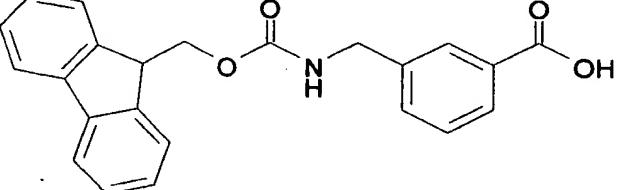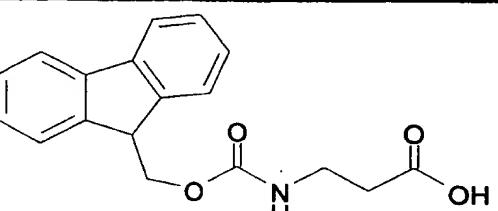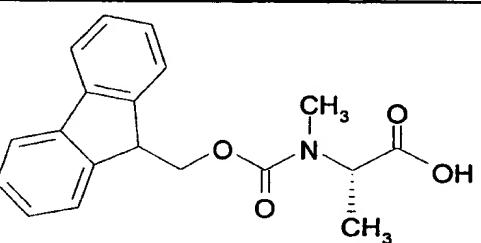
F		239mg
G		199 mg
H		208 mg

General Procedure for the Acylation Step with acids

To each of 4 vessels containing 16 Irori MicroKan™ containers (each container containing 10 mg, 0.004 mmol resin 9) in DMF (30 ml) was added a solution of PyBOP (333 mg, 0.64 mmol), DIPEA (224 µl, 1.28 mmol) and one of the acids (see table 3 for amounts) in DMF (5 ml) and the mixture shaken for 16 h. The vessels were then drained and the MicroKan™ containers washed with DMF, DCM, DMF, DCM, and Et2O (each 3 times).

R3		
I		107 mg
J		74mg

K	(structure: methylbenzoic acid, H_3C substituted benzene with $COOH$)	96 mg
L	(structure: 1-methylpyrrole-2-carboxylic acid, $N-CH_3$ pyrrole with $COOH$)	80 mg

General Procedure for the analytical cleavage of resin 10

The resin 10 is treated with a 10% solution of *meta*-chloro-perbenzoic acid (mcpba) in DCM for 4 h. The solution is then removed by filtration and the beads washed with DCM. A single bead from each 64 MicroKan™ container is then treated with a 10% solution of 1-methylpiperazine in DMSO and left to stand for 16 h. The resulting cleaved solution is then subjected to analysis by electrospray mass spectrometry. The mass spectra for the cleaved analytical constructs are shown in the spectra identified in the column headed "Construct Cleavage" in the table below. The mass spectra for the first four monomer combinations are shown in Figures 11 to 14.

General Procedure for the photocleavage of resin 10

A single bead from the each 64 MicroKan™ container is swelled in DMSO (containing 0.2% H_2NNH_2) and then subjected to photolysis for 4 h and the solution subjected to analysis by electrospray mass spectrometry. The mass spectra of the photolysis cleavage products of the library are shown in the spectra identified in the column headed "UV Cleavage" in the table below. The mass spectra for the first four monomer combinations are shown in Figures 15 to 18.

Results

Monomer Combinations			Mass Spec ID	
			UV Cleavage	Construct Cleavage
A	I	E	L993489	L991907
A	I	F	L993490	L991908
A	I	G	L993491	L991909
A	I	H	L993492	L991910
A	J	E	L993493	L991911
A	J	F	L993494	L991912
A	J	G	L993495	L991913
A	J	H	L993496	L991914
A	K	E	L993497	L991915

A	K	F	L993498	L991916
A	K	G	L993499	L991917
A	K	H	L993500	L991918
A	L	E	L993501	L991919
A	L	F	L993502	L991920
A	L	G	L993503	L991921
A	L	H	L993504	L991922
B	I	E	L993505	L991923
B	I	F	L993506	L991924
B	I	G	L993507	L991925
B	I	H	L993508	L991926
B	J	E	L993509	L991927
B	J	F	L993510	L991928
B	J	G	L993511	L991929
B	J	H	L993512	L991930
B	K	E	L993513	L991931
B	K	F	L993514	L991932
B	K	G	L993515	L991933
B	K	H	L993516	L991934
B	L	E	L993517	L991935
B	L	F	L993518	L991936
B	L	G	L993519	L991937
B	L	H	L993520	L991938
C	I	E	L993521	L991939
C	I	F	L993522	L991940
C	I	G	L993523	L991941
C	I	H	L993524	L991942
C	J	E	L993525	L991943
C	J	F	L993526	L991944
C	J	G	L993527	L991945
C	J	H	L993528	L991946
C	K	E	L993529	L991947
C	K	F	L993530	L991948
C	K	G	L993531	L991949
C	K	H	L993532	L991950
C	L	E	L993533	L991951
C	L	F	L993534	L991952
C	L	G	L993535	L991953
C	L	H	L993536	L991954
D	I	E	L993537	L991955
D	I	F	L993538	L991956
D	I	G	L993539	L991957
D	I	H	L993540	L991958
D	J	E	L993541	L991959
D	J	F	L993542	L991960
D	J	G	L993543	L991961
D	J	H	L993544	L991962
D	K	E	L993545	L991963
D	K	F	L993546	L991964
D	K	G	L993547	L991965
D	K	H	L993548	L991966

| D	L	E	L993549	L991967
D	L	F	L993550	L991968
D	L	G	L993551	L991969
D	L	H	L993552	L991970

As can be seen from the mass spectra shown in Figures, when the constructs are cleaved photolytically at the second cleavage site to release substrate, the absence of any readily ionisable group on the substrate means that the mass spectrum does not show a strong molecular ion and hence identification of the substrate is difficult. However, when the constructs to the bead are cleaved at the first cleavage site to release an analytical fragment containing the substrate and sensitising and peak splitting groups, a strong characteristic and readily identifiable split molecular ion is produced, thereby enabling the substrate to be identified. In most cases, the identity of the substrate from the molecular ion in the mass spectrum correlated with the identity predicted from the known synthesis history of the resin beads in each MicroKan™. However, in certain of the reactions, the substrates formed oxidation products under the cleavage conditions used to unlock the safety catch linker but such oxidation products could be readily identified as M+16 ions in the mass spectrum. Thus a further advantage of the constructs of the invention is that it provides a means of determining when a particular reaction has not proceeded according to plan.

The dual linker constructs of the invention allow cleavage to take place either to release an analytical construct containing a mass spectrometric sensitising group, or to release substrate. Thus, the constructs enable QC procedures to be carried out at the single bead level in a manner that would not have been possible if the constructs had contained only a single linker and no mass spectrometric sensitising group.

EXAMPLE 7
Preparation of a Construct Containing an Alloc Linker at the First Cleavage site

An example of a construct containing an allyloxycarbonyl ("Alloc") linker at the first cleavage site and an acid labile Rink linker at the second cleavage site was prepared according to the reaction scheme shown in Scheme 9. In this example, palladium (0) is used to cleave the allyloxy carbonyl group from the amino group of the diamino ethane group so as to release a free amino group that can then function as a mass spectrometric sensitising group.

60
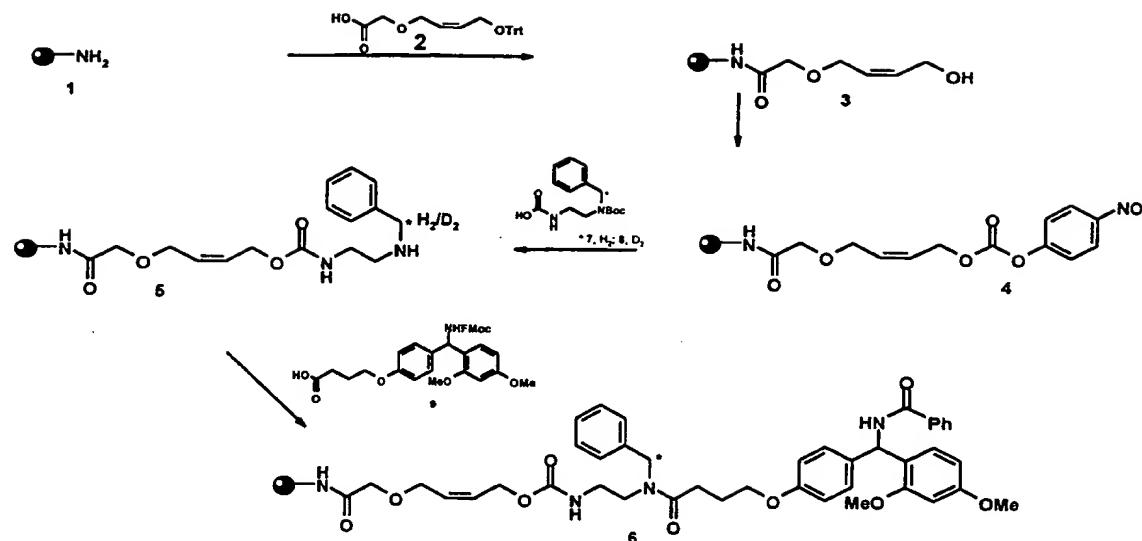
Scheme 9

Experimental for Scheme 9

General

In this section, the compound numbers refer to the compound numbers in Scheme 9.

All washings were carried out fivefold with the quoted solvents. Standard washing procedure A: DMF, DCM, DMF, DCM, ET2O, DCM and Et_2O, all five fold each.

Commercially available acid **2** (65 mg, 0.115 mmol) (Neosystems) was dissolved in DMF (0.5ml) and to this added DIPEA (40 μl, 0.23 mmol) followed by HATU (44 mg, 0.115 mmol). This mixture was allowed to stand for 5 mins and then added to commercially available Argogel NH2 (Resin 1) (50 mg, 0.023mmol), having an average bead diameter of 150 μm and a loading of 0.46 mmol g^{-1}, and shaken for 2 h. The resin was then drained and washed according to standard washing procedure A and then dried *in vacuo*. The resin was then treated with a solution of DCM, TFA and TES (1 ml, 95:2:5 respectively) for 2h and then washed and drained as above and then this treatment with DCM, TFA and TES was repeated for 1 h. The resin was washed and drained as according to standard washing procedure A to give resin **3**.

Resin 3 (50 mg, 0.023 mmol) was then treated with a solution of 4-nitrophenylchloroformate (23mg, 0.115 mmol) in DCM (1 ml) and shaken for 16h. The resin was then quickly drained and washed with DCM and Et2O to give resin 4. To this was added directly a solution of amines 7 (50 mg, 0.2mmol) and 8 (50 mg, 0.2mmol) and DIPEA (70 □l, 0.4 mmol) in DCM (1ml) and shaken for 16 h. The resin was then drained and washed according to standard washing procedure A and then dried in vacuo. This resin was then treated with a solution of 20% TFA in DCM (1 ml) and shaken for 2h. The resin was drained, washed with a solution of 10% DIPEA in DMF (2 ml) and then drained and washed according to standard washing procedure A and then dried in vacuo to give resin 5.

Rink acid 9 (65 mg, 0.115 mmol) was dissolved in DMF (0.5 ml) and DIPEA (40 □l, 0.23 mmol) and HATU (44mg, 0.115 mmol) added and this mixture allowed to stand for 5 min. This solution was then added to resin 5 (25 mg, 0.012 mmol) and shaken for 2h. . The resin was washed and drained as according to standard washing procedure A and dried in vacuo. This resin was then treated with a 20% solution of piperidine in DMF (1 ml) for 1 h. The resin was washed and drained as according to standard washing procedure A and dried in vacuo. Benzoic acid (14 mg, 0.115 mmol) was dissolved in DMF (0.5 ml) and DIPEA (40 □l, 0.23 mmol) and HATU (44mg, 0.115 mmol) added and this mixture allowed to stand for 5 min. This solution was then added to the resin and shaken for 2h. The resin was washed and drained as according to standard washing procedure A and dried in vacuo to give resin 6

Cleavage of Resin 6: Method 1

Resin 6 (0.5 mg) was swelled in DCM and then $(Ph_3P)_4Pd(0)$ (2.7 mg, $2.3E^{-3}$ mmol) added followed by morpholine (10ml, 0.11 mmol) and the mixture shaken for 1 h. A sample of the liquid was removed and analysed by electrospray mass spectrometry to give an analysis as shown in figure 17. As with the previous examples, the presence of a mass spectrometric sensitising group and a peak splitting group enables a strong molecular ion to be formed and to be readily identified.

Cleavage of Resin 6: Method 2

Resin 6 (0.5 mg) was swelled in a mixture of DMSO, THF and 0.5M HCl (0.5 ml, 2:2:1 respectively) and then $(Ph_3P)_4Pd(0)$ (2.7 mg, $2.3E^{-3}$ mmol) added followed by morpholine (10ml, 0.11 mmol) and the mixture shaken for 1 h. A sample of the liquid was removed and analysed by electrospray mass spectrometry to give an analysis as shown in figure 18.

EXAMPLE 8
Preparation of a Construct Containing an Anthracenyl UV Chromophore

In order to demonstrate that UV spectrometry can be used to provide accurate quantitative information about the products of solid phase reactions, a construct containing an anthracenyl UV chromophore was prepared according to the synthetic route shown in Schemes 10, 11 and 12 below. The construct was provided with a sulphonamide linker at the first cleavage site and a Rink linker at the second cleavage site, an ethylene diamine chain connecting the two linkers and the two nitrogen atoms of the diamine chain serving as the points of attachment of the UV chromophore and either a peak splitting deuterium labelled benzyl group or an unlabelled benzyl group.

Scheme 10 below illustrates the synthesis of a non-resin bound intermediate construct, Scheme 11 illustrates the attachment of the construct of Scheme 10 to a resin support and subsequent reactions on the resin, and Scheme 12 illustrates the attachment of different substrate groups to the constructs and subsequent cleavage experiments. In the experimental section below, the compound numbers used relate to the compounds identified in Schemes 10, 11 and 12.

63

Scheme 10

5
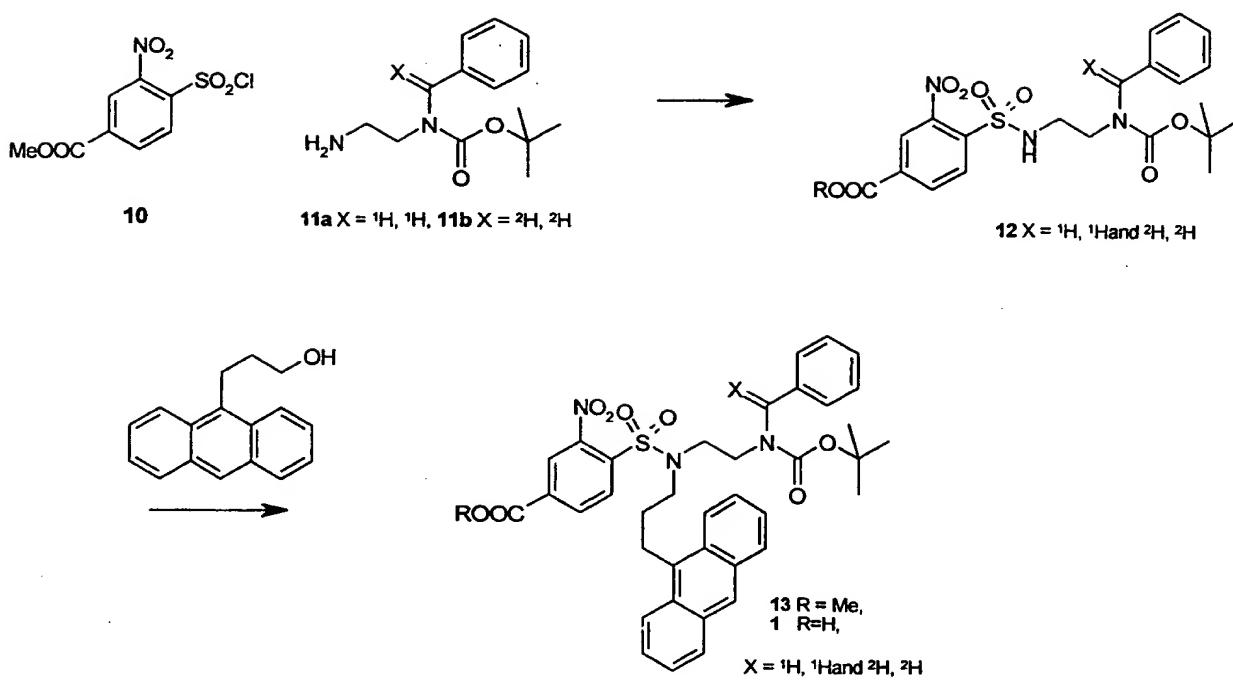
Experimental for Scheme 10

10

12 X = ¹H, ¹Hand ²H, ²H
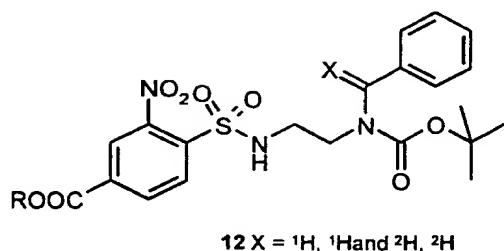
Preparation of Compound 12 (X = ¹H, ¹H)

15   Dry triethylamine (25 µl, 0.2 mmol) was added to a solution of the amine (X=H, H)
(11) (0.05 mmol) in dry dichloromethane (1 ml).   A solution of 2-nitro-4-

methylbenzoatesulfonyl chloride (10) (25 mg, 0.1 mmol) in dry dichloromethane (0.5 ml) was then added dropwise and the mixture stirred for 15 minutes. The solvent was evaporated *in vacuo* and the residue purified by flash chromatography using 2:1 hexane/ethyl acetate as eluent to afford the sulphonamide (12) (23.3 mg, 95%) as a colourless oil, R_f 17/50 [hexane-ethyl acetate (2:1)]; (Found: C, 53.7; H, 5.4; N, 8.4; S, 6.5. $C_{22}H_{27}N_3O_8S$ requires C, 53.5; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.38 (9H, s, H-18), 3.10 (2H, t, J 8, H-9), 3.24 (2H, t, J 8, H-10), 3.95 (3H, s, O-Me), 4.32 (2H, s, H11), 7.13-7.32 (5H, m, H-13, 14 and 15), 8.09 (1H, d, J 8, H-6), 8.29 (1H, s, H-3), 8.32 (1H, d, J 8, H-3); δ_C (DMSO-d^6) 28.3 (C-18), 40.2 (C-9 and 10), 46.2 (C-11), 53.2 (O-Me), 79.3 (C-17), 125.1 (C-13), 127.2 (C-14 and 15), 128.6 (C-6), 130.3 (C-13), 133.1 (C-5), 134.4 (C-12), 136.6 (C-1), 147.6 (C-4), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 494 (M-H)⁻; HPLC, R_t 6.8 min.

Preparation of Compound 12 (X = ²H, ²H)

Dry triethylamine (25 μl, 0.2 mmol) was added to a solution of the amine (X=²H, ²H) (11) (0.05 mmol) in dry dichloromethane (1 ml). A solution of 2-nitro-4-methylbenzoatesulfonyl chloride (10) (25 mg, 0.1 mmol) in dry dichloromethane (0.5 ml) was then added dropwise and the mixture stirred for 15 minutes. The solvent was evaporated *in vacuo* and the residue purified by flash chromatography using 2:1 hexane/ethyl acetate as eluent to afford the sulphonamide (12) (23.5 mg, 94%) as a colourless oil, R_f 17/50 [hexane-ethyl acetate (2:1)]; (Found: C, 53.2; H, 5.6; N, 8.5; S, 6.4. $C_{22}H_{25}D_2N_3O_8S$ requires C, 53.3; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.40 (9H, s, H-18), 3.05 (2H, m, H-9), 3.20 (2H, dt, J 3 and 13, H-10), 3.9 (3H, s, O-Me), 7.10-7.35 (5H, m, H-13, 14 and 15), 8.09 (1H, d, J 8, H-6), 8.35 (1H, d, J 8, H-5), 8.40 (1H, m, H-8), 8.45 (1H, s, H-3),.; δ_C (DMSO-d^6) 27.8 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 53.0 (O-Me), 79.1 (C-17), 124.8 (C-13), 127.0 (C-14 and 15), 128.3 (C-6), 130.0 (C-13), 132.9 (C-5), 134.2 (C-12), 136.4 (C-1), 147.5 (C-4), 154.7 (C-2), 163.5 (C-16 and C=O); MS (Electrospray +ve) m/z 496 (MH)⁺; HPLC, R_t 6.8 min.

65

Preparation of Compounds 13 and 1

Compound 13

X = ¹H, ¹H and ²H, ²H
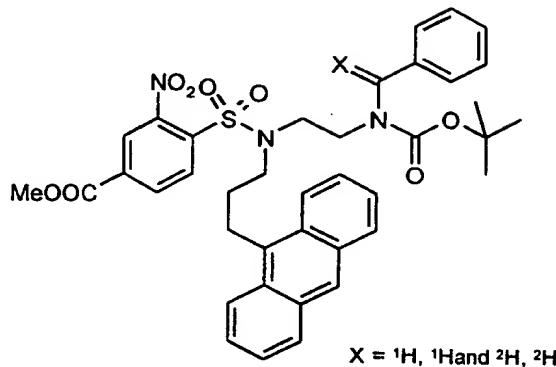
Di-*tert*-butylazodicarboxylate (32.6 mg, 142 μmol) was dissolved in dry
tetrahydrofuran (0.5 ml) under nitrogen and added dropwise to a stirred solution of
triphenylphosphine (37.1 mg, 142 μmol), 3-(9-anthracene)propanol (17.6 mg, 74 μmol) and
sulphonamide **12** (X=H, H and ²H, ²H) (35 mg, 71 μmol), dissolved in dry tetrahydrofuran
(1 ml) under nitrogen. After one hour the reaction was deemed complete by tlc and the
solution was evaporated to near-dryness. The residue was purified by flash chromatography

X = ¹H, ¹H and ²H, ²H
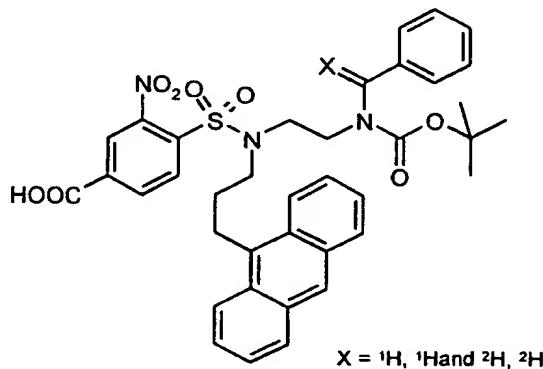
Compound 1

using 2:1 hexane/ethyl acetate containing 10% triethylamine to afford the sulphonamide **13**
(45 mg, 90%) as a colourless oil, R$_f$ 15/38 (hexane-ethyl acetate (2:1)); MS (Electrospray
+ve) m/z 612, 614 (MH-Boc)$^+$, MS (Electrospray –ve) m/z 756, 758 (M-H+formate) HPLC,

66

R$_t$ 6.9 min. 2M aqueous sodium hydroxide (0.35 ml, 0.7 mmol) was added dropwise to a solution of the ester 13 (0.25 g, 0.35 mmol) in methanol-1,4-dioxane (4:1) (2 ml). After stirring at room temperature for 1 hour (further methanol-dioxane (ca. 1 ml) was added as necessary to ensure a single phase) sufficient 2M HCl added to render the solution mildly

5   acidic. The solution transferred to a separating flask containing ethyl acetate (50 ml) and water (10 ml) and after partition the aqueous layer was extracted again with ethyl acetate (25 ml). The combined organic extracts were dried (MgSO$_4$) and evaporated under reduced pressure to afford the acid 1 (0.23 g, 94%) as a colourless oil; R$_F$ 2/40 [hexane-ethyl acetate (2:1)]; δ_H (CDCl$_3$) 1.40 (9H, br. d, tBu), 1.90 (2H, br. m, CH$_2$), 3.3-3.7 (8H, br. m, 4xCH$_2$),

10   7.10-8.30 (17H, br. m, Arom. H); MS (electrospray +ve) m/z 697, 699 HPLC, R$_t$ 7.2 min.
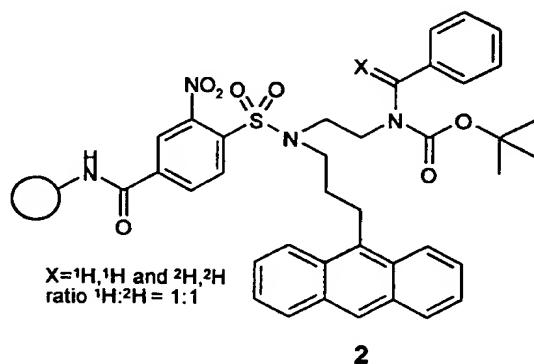
Scheme 11

a: R=   b: R=   c: R=   d: R=

15

Scheme 12

5

X=¹H,¹H and ²H,²H
ratio ¹H:²H = 1:1   3

7

a: R= [structure with NMe₂]

c: R= [structure]

d: R= [structure]

6a,c,d

8a,c,d

10
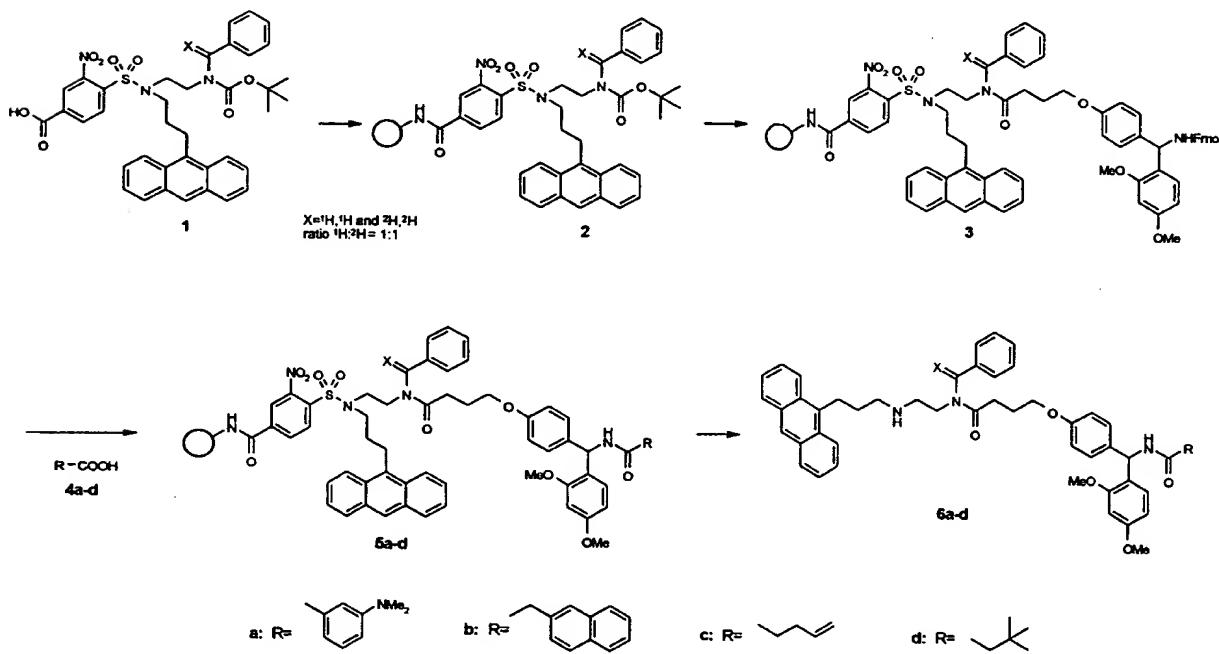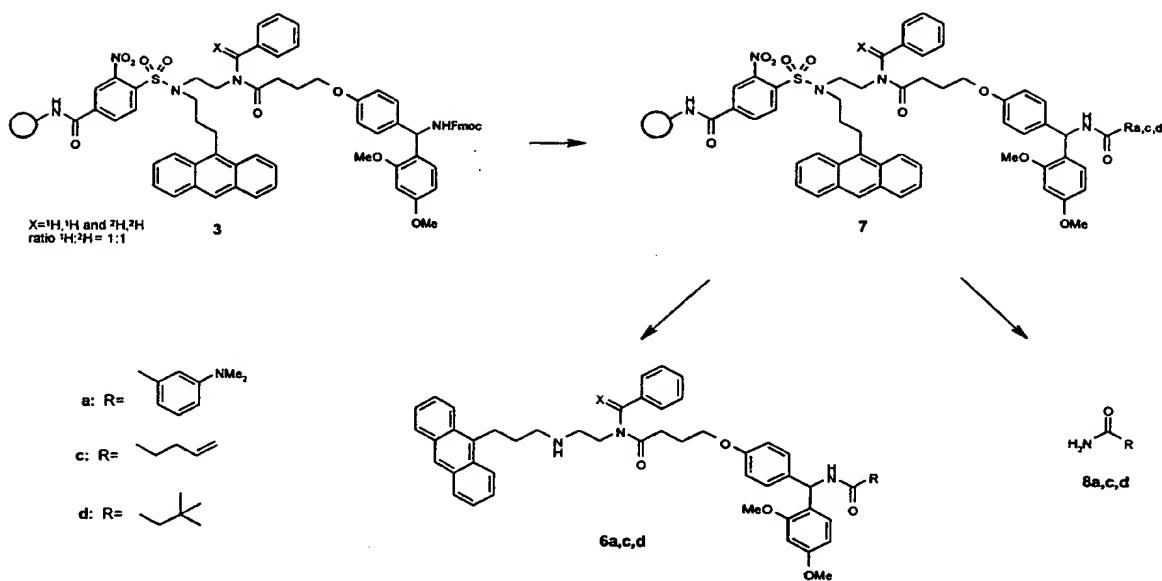
Experimental for Schemes 11 and 12

X=¹H,¹H and ²H,²H
ratio ¹H:²H = 1:1

2

Dry aminomethyl Argogel® (0.87 g, 0.35 mmol)), having an average bead

15   diameter of 150 μm and a loading of 0.4 mmol g⁻¹, in a reaction vessel flushed with dry

nitrogen was swollen with dry dichloromethane (5 ml). In a separate flask flushed with

68

nitrogen, the benzoic acid (1) (0.67 g, 0.93 mmol), PyBOP (0.728 g, 1.4 mmol) and 1-hydroxy-benzotriazole (0.19 g, 1.4 mmol) were dissolved in dry dimethylformamide (2 ml)and the resulting solution stirred two minutes before being transferred to the suspension of resin *via cannula*. A slow stream of nitrogen was aspirated through the solution for five

5   minutes to ensure mixing and diisopropylethylamine (0.49 ml, 2.8 mmol) then added. Agitation was continued for 18 h. whereupon the resin (2) was drained, washed (dichloromethane (3x7 ml), dimethylformamide (3x7 ml) and dichloromethane (3x7 ml)) and dried *in vacuo*. An analytical sample of 2 gave a negative Kaiser test.

3

10
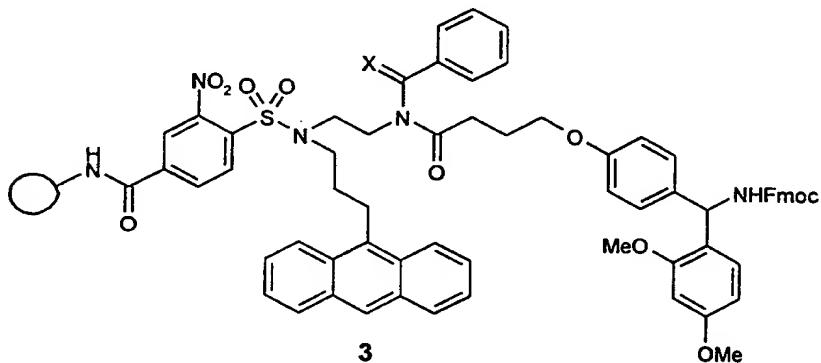
Resin 2 (0.35 mmol) was swollen in dichloromethane (3 ml) and phenol (66 mg, 0.7 mmol) added, followed by trifluoroacetic acid (4.5 ml). The resin was gently agitated for 5 minutes using a stream of nitrogen then drained and washed with dichloromethane

15   (2x5 ml). The process was repeated and, after draining, the resin washed (dichloromethane (3x8 ml), 10% diisopropylamine in dichloromethane (2x6 ml) and dichloromethane (4x8 ml)) and dried *in vacuo*. An analytical sample gave a positive Kaiser test.

The resin, in a reaction vessel flushed with nitrogen, was swollen with dry

20   dichloromethane (5 ml). In a separate flask flushed with nitrogen C-4 RINK acid (0.795 g, 1.4 mmol), PyBOP (1.46 g, 2.8 mmol) and 1-hydroxybenzotriazole (0.378 g, 2.8 mmol) were dissolved in dry dimethylformamide (3 ml) and the resulting solution transferred to the suspension of resin *via cannula*. This mixture was agitated gently by aspirating a stream of nitrogen through the solution and after five minutes dry diisopropylethylamine

25   (0.98 ml, 5.6 mmol) was added. Agitation was continued on a mechanical shaker for 18 h. whereupon the resin (3) was drained and washed (dichloromethane (3x8 ml),

69

dimethylformamide (3x5 ml) and dichloromethane (3x5 ml)) and dried *in vacuo*. An analytical sample gave a negative Kaiser test.

5a-d
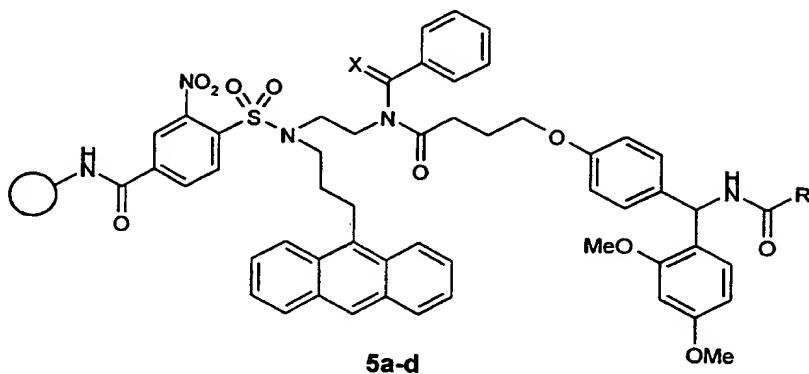
A sample of resin **3** (50 mg, 14 µmol) was treated with 20% v/v piperidine in 1:1 dichloromethane/dimethylformamide (2 ml) for 15 minutes. The resin was drained and washed (dichloromethane (3x2 ml), dimethylformamide (3x2 ml) and dichloromethane (3x2 ml)) then dried *in vacuo*, an analytical sample giving a positive bromophenol blue test. The reaction vessel containing the resin was flushed with nitrogen and dry 1:1 dichloromethane /dimethylformamide (0.5 ml) added. In a separate flask flushed with nitrogen PyBOP (57 mg, 110 µmol), 1-hydroxybenzotriazole (15 mg, 110 µmol) and the appropriate acid (**4a-d**) (55 µmol) were dissolved in dry 1:1 dichloromethane-dimethylformamide (0.5 ml) and this solution added in one portion to the suspension of resin, through which a stream of nitrogen was aspirated to ensure uniform mixing. After agitating for 18 h. the resin was drained, washed (dichloromethane (3x2 ml), dimethylformamide (4x2 ml) and dichloromethane (3x2 ml)) and dried *in vacuo* to give resins **5a-d**. An analytical sample of each gave a negative Kaiser test.

A small sample of each of the four resins was treated with DBU/ mercaptoethanol in acetonitrile to release **6a-d** which were analysed by HPLC and low-resolution mass spectrometry:

6a (R = 3-dimethylaminobenzoyl) HPLC R_t 4.51 min., MS (electrospray +ve) *m/z* 843.7 and 845.7;

6b (R = 2-naphthylacetoyl) HPLC R_t 5.41 min., MS (electrospray +ve) *m/z* 863.4 and 865.4;

6c (R = 4-pentenoyl) HPLC R$_t$ 4.98 min., MS (electrospray +ve) *m/z* 778.7 and 780.7;

6d (R = *tert*-Butylacetoyl) HPLC R$_t$ 5.18 min., MS (electrospray +ve) *m/z* 795.0 and 797.0.

<u>**Relative quantitation.**</u>

5   Approximately equal quantities (ca. 50 mg) of resins **5a** and **5b** were combined and treated with 4-mercaptobenzoic acid (43 mg, 20 µmol) and morpholine (49 µmol) in dichloromethane (2 ml). After one hour the solution was drained and the resin washed with dichloromethane (2x1 ml). These combined washings were diluted with ethyl acetate (10 ml) and washed firstly with 50% saturated aqueous sodium bicarbonate (3 ml), water (3 ml)

10   and brine (2 ml) and dried over magnesium sulphate. The filtered solution was evaporated to afford approximately 5 mg of a mixture of **6a** and **6b**. The ratio of components in this mixture was assessed firstly by HPLC, measuring peak areas at 254 nm and 386 nm, which was correlated with the ratio determined from the magnitude of the integrations of characteristic resonances in the ^{1}H NMR spectrum.

15

HPLC analysis

	6a	**6b**
Area ratio (254 nm)	1.00	1.30
Area ratio (386 nm)	1.00	1.26

^{1}H nmr analysis

	6a	**6b**
Ratio	1.00	1.33

20

        In the same way a mixture of resins **5c** and **5d**, afforded a mixture of **6c** and **6d**, the ratio of which were again assessed by HPLC and correlated with the ratios measured using ^{1}H nmr spectroscopy.

25   **HPLC analysis**

	6c	**6d**
Area ratio (254 nm)	1.00	0.93
Area ratio (386 nm)	1.00	0.97

¹H nmr analysis

	6c	6d
Ratio	1.00	0.96

Finally, **6a**, **6b**, **6c** and **6d** were combined in one solution, which was analysed using HPLC and ¹H nmr as before:

HPLC analysis

	6a	6b	6c	6d
Area ratio (254 nm)	0.87	1.35	1.00	1.00
Area ratio (386 nm)	0.84	1.30	1.00	0.99

¹H nmr analysis

	6a	6b	6c	6d
Ratio	0.85	1.34	1.00	1.05

Resin 3 (214 mg, 59 µmol) was treated with 20% v/v piperidine in 1:1 dichloromethane/dimethylformamide (3 ml) for 60 minutes. The resin was drained, washed (dichloromethane (3x3 ml), dimethylformamide (3x3 ml) and dichloromethane 93x3 ml)) and dried *in vacuo*, an analytical sample giving a positive bromophenol blue test. The reaction vessel was then flushed with nitrogen and the resin swollen with dry 1:1 dichloromethane/dimethylformamide (2 ml).

In a separate flask flushed with nitrogen PyBOP (245 mg, 470 µmol), 1-hydroxybenzotriazole (64 mg, 470 µmol) and a mixture of 4-pentenoic acid (6.0 µl, 59 µmol), 3-dimethylaminobenzoic acid (19.5 mg, 120 µmol) and *tert*-butylacetic acid (23 µl, 180 µmol) were dissolved in dry 1:1 dichloromethane/dimethylformamide (1 ml) and the solution added in one portion to the suspension of resin. A gentle stream of nitrogen was passed through the mixture and after five minutes diisopropylethylamine (165 µl, 940 µmol) was added. After agitating for 18 h. the resin (7) was drained, washed (dichloromethane (3x4 ml), dimethylformamide (4x4 ml) and dichloromethane (3x4 ml)) and dried *in vacuo*. An analytical sample of 7 gave a negative Kaiser test.

A small sample of the resin 7 was treated with DBU/ mercaptoethanol in

72

acetonitrile to release a mixture of 6a, 6c and 6d the relative quantities of these components being assessed by HPLC, measuring the peak areas at 254 and 386 nm (table).

A single bead of 7 was then selected and cleaved in the same way. The ratio of the
5   components was again measured using HPLC.

Approximately 200 mg of resin 7 (0.056 µmol) was swollen with dichloromethane and the excess solvent drained away. Phenol (10.5 mg, 0.112 µmol) was added followed by 80% trifluoroacetic acid in dichloromethane such that this added volume constituted one
10   third of the total. After agitating for two hours the pale red solvent was drained into a round-bottomed flask and the resin washed with distilled dichloromethane (3x3 ml). The combined organics were evaporated to dryness to afford a mixture of the primary amides 8a, 8c and 8d. The ratio of these components was assessed by measuring the magnitude of the integration of characteristic resonances in the ^{1}H nmr spectrum.
15

HPLC analysis (bulk beads)

	6a	6c	6d
Area ratio (254 nm)	0.71	1.00	0.44
Area ratio (386 nm)	0.71	1.00	0.46

HPLC analysis (single bead)

	6a	6c	6d
Area ratio (254 nm)	0.71	1.00	0.42
Area ratio (386 nm)	0.69	1.00	0.41

20   **^{1}H nmr analysis**

	8a	8c	8d
Ratio	0.74	1.00	0.42

The results set out in the tables above illustrate that there is a remarkably good correlation between the ratios obtained by the UV method and the ratios obtained by the
25   well established and proven method of nmr analysis. Thus, the data demonstrate that UV detection and analysis can be used to provide a very accurate means of giving quantitative

information about the reaction products of solid phase syntheses. Of particular significance is the fact that UV analysis has been shown to provide an accurate means of providing quantitative data even at the single bead level where the amounts of compound available for analysis are of the order of only 1 nanomole.

5

EXAMPLE 9

Preparation of a Construct Containing a Dansyl Group as a UV Chromophore

A second UV chromophore-containing construct was prepared, but this time
10   including a dansyl group as the chromophore rather than the anthracenyl group. The sequence of steps leading to the construct is shown in Scheme 13 below. As can be seen from Scheme 13, the construct is analogous to the construct of Example 8 in that a sulphonamide linker group provides a first cleavage site to enable release of analytical fragment, and a Rink linker provides a second cleavage site to enable release of substrate.
15   However, in this example, the chromophore, rather than being linked to the sulphonamide nitrogen, is attached to a lysine group positioned between the ethylene diamine chain and the Rink linker .

74

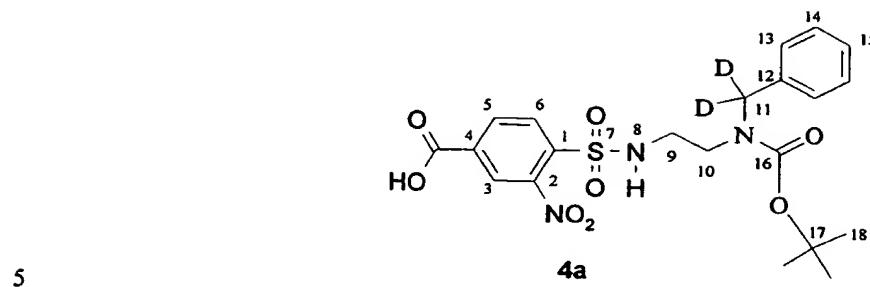## Scheme 13

5
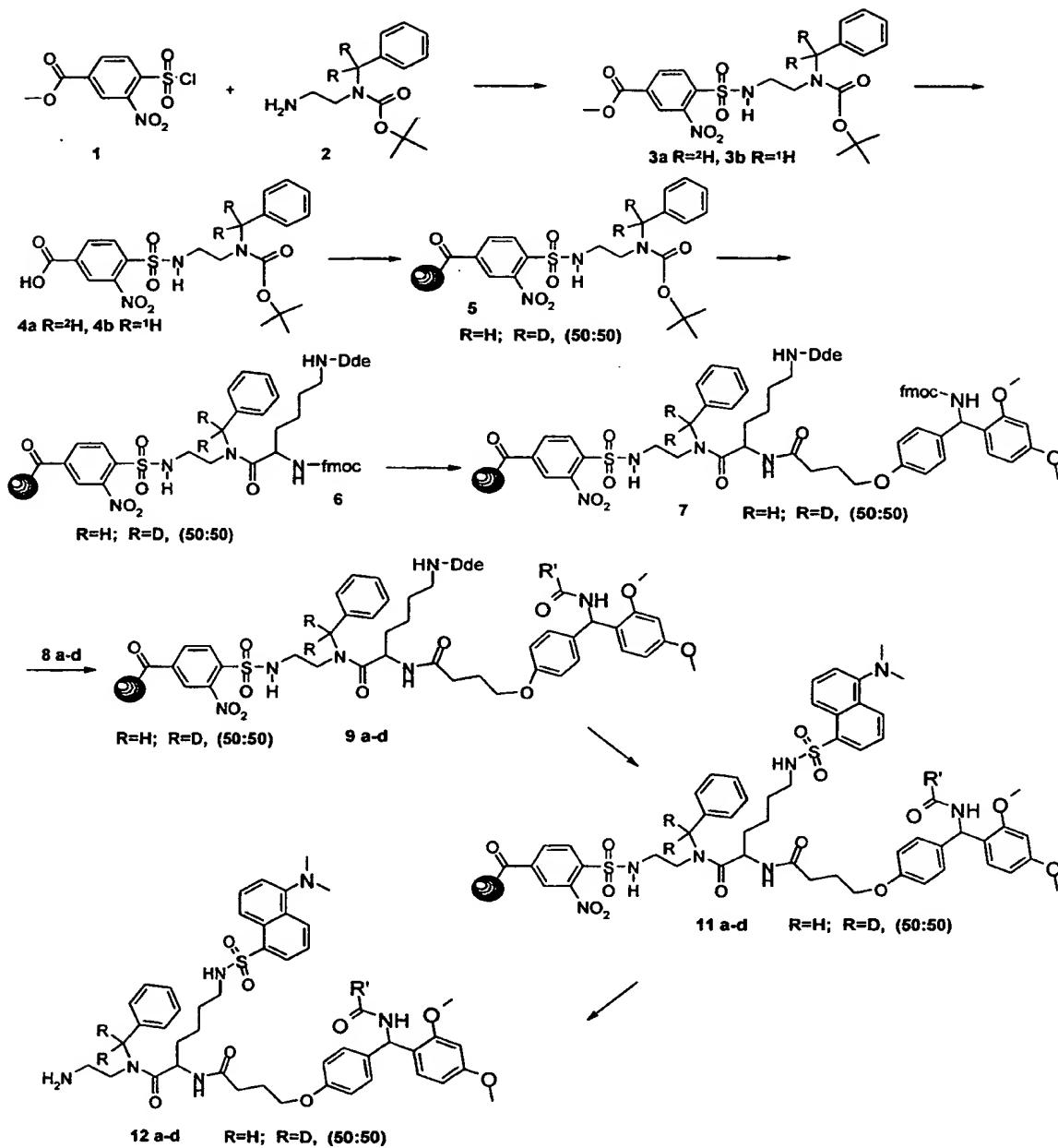
Experimental for Scheme 13

10

In the following experimental details, the compound or construct numbers refer to the compounds and constructs identified in Scheme 13.

Preparation of Compound 3a

3a

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride **1** (1.10 g, 3.90 mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2a** (1.00 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 2 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished *the sulfonamide 3a* (1.53 g, 79%) as white flakes; mp 129-130°C; R_f 0.31 [MTBE:hexane (1:1)]; (Found: C, 53.2; H, 5.6; N, 8.5; S, 6.4. $C_{22}H_{25}D_2N_3O_8S$ requires C, 53.3; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.40 (9H, s, H-18), 3.05 (2H, m, H-9), 3.20 (2H, dt, J 3 and 13, H-10), 3.9 (3H, s, O-Me), 7.10-7.35 (5H, m, H-13, 14 and 15), 8.09 (1H, d, J 8, H-6), 8.35 (1H, d, J 8, H-5), 8.40 (1H, m, H-8), 8.45 (1H, s, H-3),; δ_C (DMSO-d^6) 27.8 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 53.0 (O-Me), 79.1 (C-17), 124.8 (C-13), 127.0 (C-14 and 15), 128.3 (C-6), 130.0 (C-13), 132.9 (C-5), 134.2 (C-12), 136.4 (C-1), 147.5 (C-4), 154.7 (C-2), 163.5 (C-16 and C=O); MS (Electrospray +ve) m/z 496 (MH)$^+$; HPLC (Method A), R_t 6.07 min.

Preparati n of Compound 4a

4a

5

The ester **3a** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The

10   reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to *the acid 4a* (0.34 g, 88%) as a white solid; mp 143-145°C; R_F 0.58 [MTBE:hexane (1:1)];

15   δ_H (DMSO-d^6) 1.29 (9H, s, H-18), 3.02 (2H, m, H-9), 3.20 (2H, dt, *J* 3 and 13, H-10), 7.10-7.40 (5H, m, H-13, 14 and 15), 8.06 (1H, d, *J* 8, H-6), 8.28 (1H, s, H-3), 8.30 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 27.9 (C-18), 40.9 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.5 (C-13), 127.1 (C-14 and 15), 128.4 (C-6), 129.5 (C-13), 132.5 (C-5), 139.2 (C-12), 147.3 (C-1), 155.1 (C-4), 164.8 (C-16 and C=O); (Found: MH$^+$, 482.156364 $C_{21}H_{23}D_2N_3O_8S$ requires

20   MH+, 482.156615); HPLC (Method A), R_t 5.65 min.

Preparation of Compound 3b

25
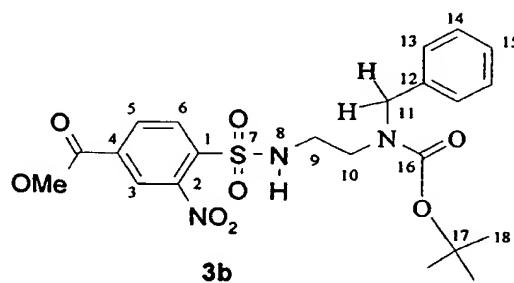
3b

To a stirred solution of 2-nitro-4-methylbenzoatesulfonyl chloride 1 (1.10 g, 3.90 mmol) and triethylamine (0.39 g, 3.90 mmol) in dry dichloromethane (40 ml), was added at 0°C the amine **2b** (0.95 g, 3.9 mmol) in dry dichloromethane (20 ml) over 30 min. The reaction mixture was stirred for a further 3 h at room temperature then concentrated *in vacuo* to give a yellow oil. Purification by chromatography with [MTBE:hexane (1:1)] as eluant followed by recrystallisation from MTBE (5 ml) and hexane (2 ml) furnished *the sulfonamide 3b* (1.51 g, 79%) as white flakes; mp 131-132°C; R_f 0.28 [MTBE:hexane (1:1)]; (Found: C, 53.7; H, 5.4; N, 8.4; S, 6.5. $C_{22}H_{27}N_3O_8S$ requires C, 53.5; H, 5.5; N, 8.5; S, 6.5%); δ_H (DMSO-d^6) 1.38 (9H, s, H-18), 3.10 (2H, t, *J* 8, H-9), 3.24 (2H, t, *J* 8, H-10), 3.95 (3H, s, O-Me), 4.32 (2H, s, H11), 7.13-7.32 (5H, m, H-13, 14 and 15), 8.09 (1H, d, *J* 8, H-6), 8.29 (1H, s, H-3), 8.32 (1H, d, *J* 8, H-3); δ_C (DMSO-d^6) 28.3 (C-18), 40.2 (C-9 and 10), 46.2 (C-11), 53.2 (O-Me), 79.3 (C-17), 125.1 (C-13), 127.2 (C-14 and 15), 128.6 (C-6), 130.3 (C-13), 133.1 (C-5), 134.4 (C-12), 136.6 (C-1), 147.6 (C-4), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 492 (M-H)$^-$; HPLC (Method A), R_t 5.99 min.
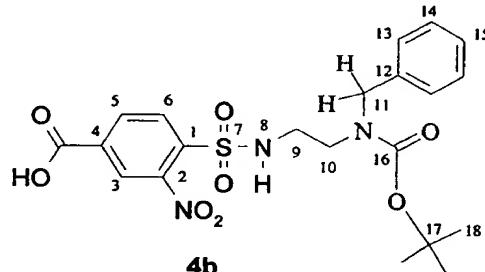
**Preparation of Compound 4b**

4b

The ester **3b** (0.40 g, 0.80 mmol) in methanol (2 ml) was treated with 1M aqueous sodium hydroxide (1.60 ml, 1.60 mmol) and stirred at room temperature for 1 hour. The reaction was then concentrated *in vacuo* and the residue dissolved in water (10 ml) then acidified with 1M hydrochloric acid (1.80 ml, 1.80 mmol) at 0°C. The resulting white precipitate was extracted with dichloromethane (3 x 10 ml). The combined organic extracts were washed with brine, dried (Na$_2$SO$_4$) and evaporated under reduced pressure to *the acid 4b* (0.36 g, 93%) as a white solid; mp 123.7-124.8°C; R_F 0.59 [MTBE:hexane (1:1)]; δ_H (DMSO-d^6) 1.42 (9H, s, H-18), 3.12 (2H, m, H-9), 3.22 (2H, m, H-10), 4.38 (2H, s, H11), 7.18-7.42 (5H, m, H-13, 14 and 15), 8.11 (1H, d, *J* 8, H-6), 8.39 (1H, d, *J* 8, H-3);

78

8.45 (1H, s, H-3); δ_C (DMSO-d^6) 27.8 (C-18), 40.1 (C-9 and 10), 45.9 (C-11), 79.1 (C-17), 124.8 (C-13), 126.9 (C-14 and 15), 128.4 (C-6), 129.9 (C-13), 132.9 (C-5), 135.8 (C-12), 137.9 (C-1), 147.5 (C-4), 153.9 (C-2), 163.7 (C-16 and C=O); MS (Electrospray -ve) m/z 478 (M-H)⁻; HPLC (Method A), R$_t$ 5.56 min.

Preparation of Compound 5

R=H; R=D, (50:50)   5
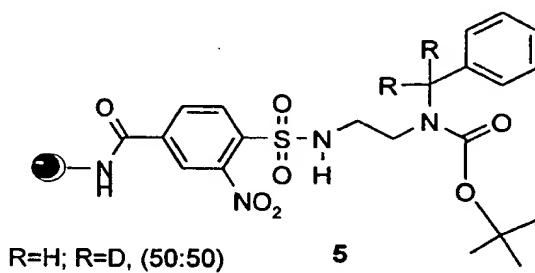
To a suspension of amine resin (Argogel-NH₂) (0.80 g, 0.32 mmol)), having a bead diameter of 150 μm and a loading of 0.4 mmol g⁻¹, in DMF (4 ml) and dichloromethane (4 ml) was added the benzoic acid (4a) (0.46 g, 0.96 mmol), and the benzoic acid (4b) (0.46 g, 0.96 mmol), followed by PyBOP (0.99 g, 1.92 mmol) and HOBT (0.26. g, 1.92 mmol) followed, 4 min. later by Hünig's base (0.49 g, 3.84 mmol). The reaction was agitated with nitrogen for 18 h and the resin then washed with dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10 ml) and dried *in vacuo* to give the *sulfonamide resin 5* (0.94 g, 97%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test was negative.

Preparation of Compound 6
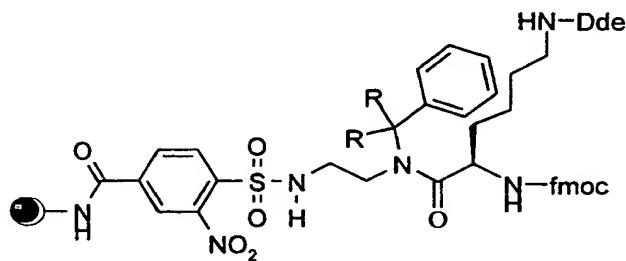
R=H; R=D, (50:50)   6

The protected resin 5 (0.85 g, 0.34 mmol) was suspended in dichloromethane (2
ml) and treated with trifluoroacetic acid (2 x 4 ml) for 5 min. Solvents were removed by
filtration and the resin washed with 10% DIPEA solution in DMF (3 x 5 ml),
dichloromethane (6 x 5 ml), DMF (6 x 5 ml), dichloromethane (6 x 5 ml), ether (2 x 5 ml).
The amine resin was then suspended in DMF (4 ml) and dichloromethane (2 ml) FMoc-
Lys(Dde)-OH (0.86 g, 1.62 mmol) was added, followed by PyBOP (0.84 g, 1.62 mmol) and
HOBT (0.22. g, 1.62 mmol). After 4 min. Hünig's base (0.42 g, 3.24 mmol) was added.
The reaction was agitated with nitrogen for 18 h and the resin then washed with
dichloromethane (6 x 10 ml), DMF (6 x 10 ml), dichloromethane (6 x 10 ml), ether (2 x 10
ml) and dried *in vacuo* to give the *sulfonamide resin 6* (0.96 g, 100%) as a yellow solid;
Kaiser test was negative; Bromophenol Blue test was negative; Resin loading 99% as
estimated by quantitative Fmoc group cleavage.

Preparation of Compound 7

R=H; R=D, (50:50)   7
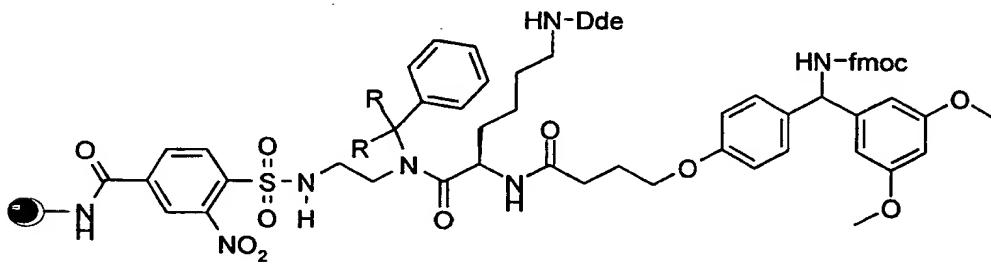
Resin (6) (0.93 g, 0.28 mmol) was treated with 20% Piperidine in DMF solution (2
x 5 ml) for 10 min. Solvents were removed by filtration and the resin washed with DMF (6
x 5 ml), dichloromethane (6 x 5 ml), and ether (2 x 4 ml). The resin was suspended in
DMF (5 ml) and dichloromethane (5 ml) and the Rink acid linker (0.92 g, 1.62 mmol) was
added, followed by PyBOP (0.84 g, 1.62 mmol) and HOBT (0.22 g, 1.62 mmol). After 4
min. Hünig's base (0.42g, 3.24 mmol) was added. The reaction was agitated with nitrogen
for 3 h and the resin then washed with dichloromethane (6 x 5 ml), DMF (6 x 5 ml),
dichloromethane (6 x 5 ml), ether (2 x 3 ml) and dried *in vacuo* to give the *sulfonamide
resin 7* (1.02 g, 98%) as a yellow solid; Kaiser test was negative; Bromophenol Blue test
was negative; Resin loading 94% as estimated by quantitative Fmoc group cleavage.

Preparation of Compounds 9a-d and 10a-d

R=H; R=D, (50:50)
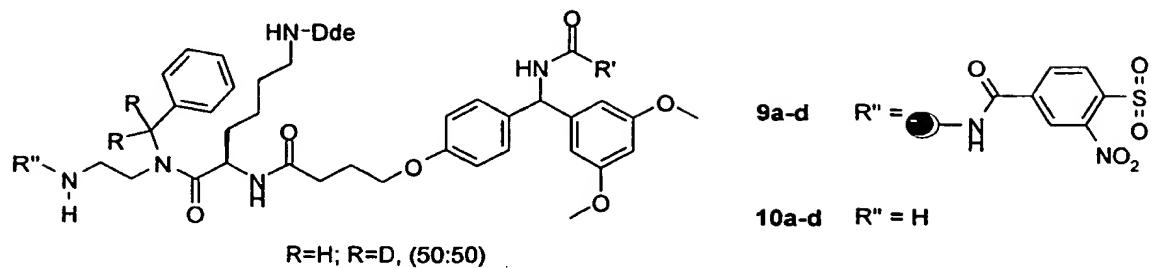
Resin (7) (70 mg, 0.02 mmol) was treated with 20% piperidine in DMF solution (2 x 1 ml) for 10 min. Solvents were removed by filtration and the resin washed with DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml). The resin was suspended in DMF (0.5 ml) and dichloromethane (0.5 ml) and the appropriate acid (8a-d) (0.15 mmol) was added, followed by PyBOP (0.08 g, 0.15 mmol) and HOBT (0.02 g, 0.15 mmol). After 4 min. Hünig's base (0.04 g, 0.30 mmol) was added. The reaction was agitated with nitrogen for 3 h and the resin then washed with dichloromethane (6 x 1 ml), DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml) and dried *in vacuo* to give the *sulfonamide resins 9a-d* as a yellow solid; Kaiser test and Bromophenol Blue test were negative in all four cases.

A small sample of each of the four resins was treated with DBU/Mercaptoethanol in acetonitrile to release *10a-d* which were analysed by low resolution mass spectrometry and RP HPLC (Method A):
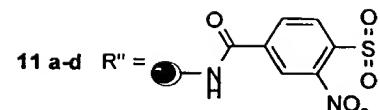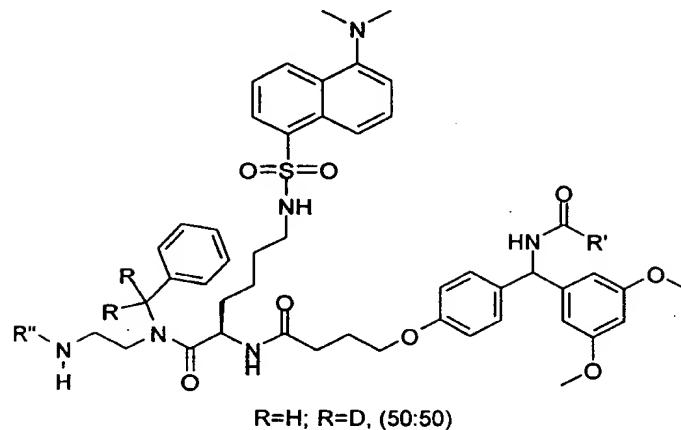
10a (R'= Z-Gly-) MS (Electrospray +ve) m/z 961 and 963 $(MH)^+$; HPLC, R_t 4.01 min.

10b (R'= Boc-Abu-) MS (Electrospray +ve) m/z 955 and 957 $(MH)^+$; HPLC, R_t 4.09 min.

10c (R'= 1-naphthylacetoyl-) MS (Electrospray +ve) m/z 938 and 939 $(MH)^+$; HPLC, R_t 4.28 min.

10d (R'= Boc-Phe-) MS (Electrospray +ve) m/z 1017 and 1019 $(MH)^+$; HPLC, R_t 4.42 min.

Preparation of Compounds 11a-d and 12a-d

R=H; R=D, (50:50)

11 a-d R" =

12 a-d R" = H

Resin **(9a-d)** (ca. 70 mg, 0.02 mmol) was treated with 10% hydrazine in DMF solution (3 x 1 ml) for 30 min. Solvents were removed by filtration and each resin washed with DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml). To each resin portion was then added dansyl chloride (40 mg, 0.15 mmol) and triethylamine (15 mg, 0.15 mmol) in dichloromethane (1 ml). After 4 h reaction time the resins were washed with dichloromethane (6 x 1 ml), DMF (6 x 1 ml), dichloromethane (6 x 1 ml), ether (2 x 1 ml) and dried *in vacuo* to give the ***sulfonamide resins 11a-d*** as a yellow solid; Kaiser test and Bromophenol Blue test were positive in all four cases.

A small sample of each of the four resins was treated with DBU/Mercaptoethanol in acetonitrile to release *12a-d* which were analysed by low resolution mass spectrometry and RP HPLC (Method B):

12a (R'= Z-Gly-) MS (Electrospray +ve) m/z 1030 and 1032 (MH)$^+$; HPLC, R$_t$ 4.77 min.

12b (R'= Boc-Abu-) MS (Electrospray +ve) m/z 1024 and 1026 (MH)$^+$; HPLC, R$_t$ 4.94 min.

12c (R'= 1-naphthylacetoyl-) MS (Electrospray +ve) m/z 1007 and 1009 (MH)$^+$; HPLC, R$_t$ 5.47 min.

12d (R'= Boc-Phe-) MS (Electrospray +ve) m/z 1086 and 1089 (MH)$^+$; HPLC, R$_t$ 5.82 min.

It can be seen from the foregoing examples that the inclusion of a UV chromophore, and mass spectrometric sensitising group into the constructs enables rapid and simple qualitative and quantitative analysis of the products of a solid phase synthesis to be undertaken by the simple and well established procedures of HPLC in combination with

5   UV detection and mass spectrometry.

The foregoing examples are intended merely to illustrate the invention and should not be construed as imposing any limitations on the scope of the claims. It will readily be apparent that numerous alternations and modifications could be made to the constructs

10   illustrated in the examples without departing from the principles underlying this invention and all such modifications and alterations are intended to be embraced by this application.